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Development in the Muslim world

NEARLY a billion of the world's inhabitants are Muslim, and of them two-thirds live in the forty-three nation-states in which Muslims form a majority. A large proportion of these nation-states have emerged since the Second World War, and they all fall into the category of developing nation, although several, of course, are at present well endowed with oil resources. The Muslim world, stretching from Morocco to Malaysia, and from Turkey to Cameroon, thus constitutes a very significant fraction of the whole developing world. With what sort of voice should it speak at the forthcoming United Nations Conference on Science and Technology for Development? Are there distinctive paths along which development should proceed? Ziauddin Sardar, of the Hajj Research Centre, King Abdul Aziz University, Jeddah, Saudi Arabia makes a strong, almost passionate case in a recent book *Science, Technology and Development in the Muslim World* (Croom Helm, £7.95) for a specifically Muslim view of development and even of science itself. No other religion would these days claim such a distinctive involvement in matters of science and technology, so Sardar's line of argument is of special interest.

The state of science, research and higher education in the Muslim world, is, it is freely admitted, woeful on almost any count. Expenditures on research and development in terms of GNP are generally small compared with those of the western world; there are few well-trained scientists and engineers; the scientific establishments are 'mediocre'; bureaucracy and corruption abound; university staff often simply teach their students to memorise and positively discourage them from regarding science as still a matter for debate and dispute. Added to this is the frequent prejudice in favour of academic work and against anything technical—only 15 per cent of Arab students, for instance, are in technical institutions.

All of this makes the Muslim world extremely vulnerable to external influences which consciously or unconsciously remould Islamic communities into subservient and inferior imitations of the developed world. Sardar uses the word 'occidental' for everything that is non-Muslim—a quaint use as the occidental world would presumably have to include India, China, much of Africa and all of South America. Be that as it may, the occidental world as properly understood, Europe and North America, is in his eyes the source of much of the trouble in the Muslim world, not just through colonial attitudes but through a willing acceptance of the occidental point of view by many of the elite in Muslim countries. Nearly a century and a half ago

Macaulay neatly summed up the position of an occidental elite in India, 'we must at present do our best to form a class who may be interpreters between us and the millions whom we govern; a class of persons, Indians in blood and colour, but English in taste, in opinion, in morals and in intellect'. Although Muslim nations now have their political independence, the occidental elite lives on, indeed is overtly encouraged in such ways as to send students to Britain so that when they return they will order British products. And the worrying thing is that these elites in almost all ways speak for their countries, deciding policy on science, technology and development. So alternative modes of development, free of a crushing reliance on industrialised nations for equipment, money and ideas, never really get a proper hearing.

Sardar sees Islamic unity as the only possible counterweight. Whatever the present gloomy picture, Islam historically produced great scientists and an entire tradition of science which integrated scientific objectivity within the Islamic world-view—a view which required that subjects of study had a social relevance. But, for the Muslim, 'all experiences are real', so metaphysics, elbowed out of Western science, has its part to play. Science illuminated by Islamic philosophy should, claims Sardar, be particularly appropriate to people's needs, and hence sensitive to a developmental route which realised potential, eliminated poverty and reduced unemployment.

Are there any signs that the Muslim world is coming to grips with this question of shaking off occidental influence when it is unhelpful? With an occidental elite entrenched it will be a difficult thing to do, but Sardar points to the return of traditional teaching of Islamic studies in some of the new technical universities such as Islamabad, Ahmed Bello, King Abdul Aziz, Kuwait, the National University of Malaysia and so on. This trend back to religious fundamentals should create an awareness amongst scientists and technologists of responsibilities to the community.

It is an interesting thesis. A cry of back to religious fundamentals and traditional modes of education in western countries would produce an uproar. And the western mind certainly has the impression that traditional Islamic practice means things such as a harsh penal code—a fear that is regularly refuelled and would need some careful dispelling. But development along the occidental model has hardly proved spectacularly successful in the Muslim world; the case for a new, or rather very old philosophical approach is certainly worth a hearing. □



X-ray health and safety

David Blow, Professor of Biophysics at Imperial College, London, offers his opinion

X-RAY crystallography provides a tool for almost every branch of science and technology. Its applications extend from the routine analysis of purity and texture of manufactured products, through the identification of unknown substances and the detection of small amounts of impurities in materials of all kinds, to fundamental investigations of physical, chemical and biological importance. This means that X-ray diffraction apparatus is in use in a great many university departments, and in much of industry.

In the UK, under the Health and Safety at Work Act 1974, all these activities will be covered by new provisions which are being drafted by the Health and Safety Executive (HSE). For a large range of routine diffraction work, especially when a closed camera is used, such as a powder camera, the modifications to existing practice will be fairly simple. But for a substantial group of research workers, they present serious problems.

One may raise three principal objections. The first is that the regulations are being proposed without regard to the level of risk. There are no records of deaths from radiation accidents involving crystallographic apparatus. There is epidemiological evidence that exposure to ionising radiation shortens life, and increases the incidence of cancer. An important contribution to this evidence comes from studies on radiologists, who early in this century were not sufficiently careful to avoid exposure. But I am not aware that any evidence has been obtained from X-ray crystallography, where considerable effort has been made to reduce background radiation to a negligible level.

The only records of identifiable injury following misuse of crystallographic equipment in the UK are of burns causing blisters (usually to the hands), or a general reddening of the skin. The incidence of blistering burns has been estimated as one per 1,000 X-ray generator-years; non-blistering burns are three or four times more common. At Imperial College, where 70 X-ray beams are currently in use, one serious burn has occurred in the last 23 years.

The price of modifications to satisfy the regulations on new X-ray apparatus will leverage £500–£1,000 per generator (with a much greater expense when additional generators are needed to minimise the problems of using one generator for several beams, or where more space is needed). Transitional costs in bringing existing X-ray equipment up to standard may be more. If the incidence of accidents is reduced to zero, an assumed lifetime of 10 years for a generator leads to an expenditure of £50,000 to £100,000 for each blistering burn which is avoided.

The second objection is that the rules have to apply equally to everybody. There is no allowance for the knowledge and experience which the professional has obtained. The law, we are told, must apply equally to all; but this principle does not apply in other contexts. Work with pathogens, or even work with inflammable substances, involves special risks which training and experience can minimise. A highly trained crystallographer may resent being forced to work in a foolproof but inconvenient fashion. There is a real danger, if the rules seem too restrictive, that crystallographers experienced enough to know the risks may indulge in some form of evasion.

The new provisions will rely heavily on an individual called the "Radiation Protection Adviser", who needs to define a scheme of work which provides a practicable method of prosecuting research, while maintaining the necessary safety standards. He must be prepared to do

battle on both sides, to maintain techniques which are safe, but to limit costs and physical complexities to a level where research may proceed without impediment. An adviser who pursues a rigid line may eliminate accidents, but will eliminate progress as well; an adviser who is inventive, and who understands the problems on both sides, may save tens of thousands of pounds—or several years—by devising better procedures and better equipment.

One anticipates a severe shortage of suitably qualified Radiation Protection Advisers, for whom there is no course of training except bitter experience. In most factories there is no comparable adviser at present. The absence of suitable advisers may represent the biggest threat to research.

No one will object to the provision of improved mechanical and electrical equipment to reduce the risk of an accident. The only worry for standard equipment is the question of cost, and its effect on the value of crystallographic work, especially if the cost is far out of proportion to the level of risk. Much more serious problems are raised for those crystallographers who deal with unconventional apparatus, especially equipment involving small apertures and monochromatising crystals which involve continual realignment, or which require constant rearrangement of components. They may feel that these regulations will make their work extremely difficult, if not impossible.

The interlocks, barriers and inconvenient schemes of work proposed in the regulations may considerably complicate the introduction of novel equipment. In a case where physical interlocks are impracticable one might, for example, have to initial each item of a long check-list on each occasion before opening the shutter. Whatever happens, work will become more cumbersome, and a complicated administrative overhead of certification and inspection will be added to the difficulties of research.

The impact on work with non-standard equipment will depend crucially on the interpretations of the law and of regulations and codes of practice. Provisions drawn up by HSE depend on an interpretation of the law. It is hard to see how that interpretation could be challenged, except to wait for regulations to be brought into effect and then to test them, if necessary, in the courts. Radiation Protection Advisers will play a crucial role in any test of the new rules.

This is apparently the first time that consultation with experts outside HSE has been attempted before regulations were drawn up. This was done informally, and the main learned societies which deal with X-ray crystallography were not approached. On the initiative of individual scientists assisting in the drafting, a very valuable meeting of the Institute of Physics was organised last summer (12 July, 1977). This was the first occasion when most crystallographers heard of the proposed regulations. Such preliminary, informal consultations are probably far more effective than "formal" ones, but definite channels for consultation with learned societies need to be established.

HSE officials, now formulating regulations, can hardly be criticised for they have a duty to produce regulations consistent with the health and safety act and with the EEC agreement known as the Euratom Directive. And they can point to the flexibility of British certification procedures. Nevertheless, a period of some months is allowed for comments to be made to the Secretary of State, after which the provisions, modified as necessary in the light of comments received, will be laid before Parliament. It is essential that diffraction workers who have views about the proposals should make good use of this period for formal consultation, involving their professional institutions and learned societies. I believe there will still be room for improvement within the legal constraints. In particular, I would like to see explicit provision for "open beam areas", where research of certain kinds could continue with the uncomplicated and safe methods which are used today. □

Radiation: how safe is "safe"?

David Dickson reports on the growing US debate over the hazards of occupational exposure to low level radiation

SERIOUS doubts are being raised in the US about the adequacy of existing safety standards for those whose work exposes them to sources of low level radiation. If substantiated, a revision of existing standards could have considerable impact on many aspects of the nuclear power industry.

The debate has been given wide public exposure by a series of Congressional hearings, held in Washington last month, which brought together three topics guaranteed to attract the attention of the average US newspaper reader: nuclear radiation, cancer, and charges of government malpractice.

Similar concerns have been raised twice before. In the 1950s medical evidence from survivors of the atomic bombs dropped on Hiroshima and Nagasaki, and from those who had worked closely with radiation for medical purposes, drew attention to the hazards of exposure to high levels of radiation. And at the end of the 1960s, fierce public controversy raged over the charge that fall-out from nuclear weapons tests had caused widespread genetic damage to unborn children.

After a period of relative dormancy the debate has now flared up again, this time over the long-term effects experienced by those known to have been exposed to what had previously been considered as acceptably-safe low levels of radiation.

In particular attention has focused on the fate of members of three particular groups: those who participated in the testing of nuclear weapons during the 1950s, those who have worked at the Government's nuclear fuel production facilities at Hanford in Richland, Washington State, and those who have worked on the production of nuclear-powered vessels, such as submarines, in the US Navy's shipyards at Portsmouth, New Hampshire.

Concern about possible long-term health effects suffered by those involved in nuclear weapons testing—as many as 300,000 military and civilian personnel were involved in tests in the Nevada Desert and the Pacific Ocean between 1945 and 1962—has been raised by reports of an abnormally high incidence of leukaemia among soldiers in one particular blast, called Smoky, which took place at Yucca Flat in 1957.

This test, which involved military manoeuvres in the test area shortly after the explosion had taken place, was specifically designed to test the

physical and psychological impact of nuclear weapons on test.

According to the Defense Department, the average exposure of men at Smoky was 1.25, considerably below the generally accepted occupational level of 5 rems a year. Yet at least eight of the 2,245 soldiers involved have since developed leukaemia, a level that has been called "out of the normal range" by the director of the Centre for Disease Control, Dr William H. Foege.

If the Smoky test has raised question about the effects of a single exposure to a relatively low amount of radiation, the other two cases—Hanford and the Portsmouth Shipyards—raise a slightly different question: the hazards associated with long-term exposure to low-level radiation sources.

Concern at the possible hazards facing workers at the Hanford plant was first raised in 1974 by Dr S. Milham, epidemiologist for the State of Washington. Indications of abnormally high incidence of deaths from certain types of cancer were confirmed by Dr Thomas Mancuso, of the Department of Epidemiology at the University of Pittsburgh, whose 13-year study of medical records revealed high incidences of multiple myeloma (cancer of the bone marrow), cancer of the pancreas, and lung cancer (*Health Physics*, Vol 33, No 5, pp 369-384).

Furthermore statistical analysis of Dr Mancuso's data by epidemiologist Dr Alice Stewart and statistician Dr George Kneale of the University of Birmingham, England, have led them

to suggest that the doubling dose for these cancers—the amount of radiation needed to double their incidence in a particular population—could be as low as 3.6 rads, 19 rads and 13 rads respectively, with a doubling dose for all cancers as low as 33 rads for men and 9 for women. These are considerably below generally accepted figures.

At Portsmouth, Dr Thomas Najarian, a blood specialist with the Boston Veterans Administration, carried out a survey of past dockyard workers after becoming suspicious at the death from leukaemia of one of his patients.

As a result of his study Dr Najarian claimed that the incidence of leukaemia among shipyard workers was four times higher than should have been expected. And according to a report in the *Boston Globe*, which assisted Dr Najarian in his study, the overall cancer death rate among Portsmouth nuclear workers was more than twice the national average, and substantially higher than the yards non-nuclear employees.

In each of the above cases the significance of the results—in particular whether the observed cancers can be blamed on the known exposure—is hotly debated. The effects of low level radiation is an issue on which scientific emotions run high, not least on the validity of the no-threshold, linear-response model which, though remaining a hypothesis, is now widely assumed in calculations of acceptable levels of risk.

Based as they are on uncertain scientific premises, Government policies can quickly become a morass. The latest victim, the exposure of whose floundering at the Congressional hearings carried frequent echoes of the Watergate scandal, was the Department of Energy, responsible both for the operation of Hanford plant and for financing Dr Mancuso's study.

The hearings were held by the health and environment subcommittee of the House Committee on Interstate and Foreign Commerce. And its suspicions had been raised by the circumstances surrounding the department's decision to terminate Dr Mancuso's contract, and to transfer the research project to the Oak Ridge Associated Universities.

The department countered charges that the contract had been terminated because of the nature of the findings by pointing out that the decision had been taken before the findings were known. And in earlier correspondence, Dr James L. Liverman, acting assistant secretary for environment, claimed the reason for the transfer was 62-year-old Dr Mancuso's "imminent retirement."



The Smoky test, 31 August 1957

On learning that Dr Mancuso was not required by the University of Pittsburgh to retire before the age of 70, Dr Liverman admitted in testimony to the subcommittee that his use of the phrase had been "unfortunate, inappropriate and perhaps even in error." Rather he claimed that the reason was a critical peer review indicating "a judgement by his scientific peers that the work should be limited, terminated, or another investigator selected to be the principal investigator."

But it was a case of out of the frying pan into the fire. Representative Paul Rogers, chairman of the subcommittee, produced a summary of the reviewers' recommendations which showed that although two referees had indeed made critical comments, the other four had been positive, and the general consensus had been that the work should be continued.

Department officials were left in confusion, and no clear reason has yet emerged for the decision to terminate the contract (on which an Inspector General's report is expected shortly). One possible explanation is a conflict between the painstaking lengths Dr Mancuso felt it necessary to go to obtain sufficiently accurate data to produce meaningful results, and pressure on the department to produce arguments that could be used in public debate. "He did not come up with a system amenable to our needs in the time-frame that we had to work with," Dr Liverman told *Nature* last week.

Whatever the reason, the department's mishandling of the issue has proved embarrassing, and reinforced suspicion that it has sought to discourage research leading to distasteful findings. And similar impressions have been raised by the reactions of the Defense Department to Dr Najarian's study of the Portsmouth shipyard workers.

In reply to the articles in the *Boston Globe*, the Pentagon claimed that the study was "simplistic" in its approach, and that its conclusions were "not credible" and "do not accurately reflect the actual health conditions for employees engaged in radiation work." Admiral H. G. Rickover, architect of the "nuclear navy", claimed that "from the best scientific evidence, we don't see a problem."

Last December, after publication of Dr Mancuso's report, the Navy asked the Department of Energy to initiate a study of the effects of radiation on shipyard workers. However, it was only after Congressional pressure and accusation of "stonewalling" that it agreed to let the Centre for Disease Control—an arm of the Department of Health, Education and Welfare—to

take a look at the medical records of Portsmouth workers, a study which the Navy had claimed was "redundant".

Part of the Navy's concern—indeed that of Government agencies in general—is the large amounts of compensation for which they could be liable if it is established that cancer victims were the result of exposure to nuclear materials, however low the radiation. And this could set an expensive precedent for private industry.

Admiral Rickover told the subcommittee that a medical follow-up of Portsmouth workers should be done without the "spectre" of its becoming the basis for future claims against the government. "If such a study is done for the purpose of establishing a massive claims programme, then we might as well say beforehand that the Government will distribute so many billions of dollars to all US citizens, and be done with it," he said.

Three main results have emerged from the recent public debate. The first of these is a stronger commitment—considered by many to be long overdue—by various government agencies to follow-up studies designed to establish more precisely the precise nature of the hazards of long-term exposure to low level radiation.

The Department of Energy, for example, has put into effect plans for a major survey of the medical histories of its past employees, a study which could cost up to \$30 million over the next ten years. And the Pentagon, which earlier admitted it merely had one individual spending a quarter of his time trying to contact survivors of the Smoky test, is instituting a similar study; widely-broadcast requests for information brought 10,000 calls in 2 weeks, and necessitated the installation of a 20-line switchboard.

The second result has been to raise serious questions about the propriety of government agencies bearing responsibility both for developments in the field of nuclear power, and for the health of those engaged in such developments. Notwithstanding the relatively "clean record" that such agencies are able to produce, the whole handling of the Mancuso affair (including attempts to use Dr Mancuso's earlier negative findings to refute the claims of Dr Milham on cancer levels among Hanford workers, despite Dr Mancuso's protests that such conclusions were premature) has provided many with a graphic illustration of the dangers.

Representative Rogers, for example, described the situation he had discovered in the department as being "the most disordered, unstructured mess that I've looked into in some

time", adding that "I'm not sure that I don't question the whole issue of the Energy Department conducting health work."

Finally the debate has revealed evidence that suggests a need to revise accepted guidelines for long-term, low-level radiation exposure. At present, the guidelines generally accepted are those laid down by the Federal Radiation Council in 1960, suggesting a maximum exposure of the general population—in addition to normal background radiation—of 500 millirem a year, and a maximum occupational exposure of 5 rem.

The Environmental Protection Agency is known to be looking closely at both of these figures, and is expected to suggest that at least the first should be revised downwards.

Scientific evidence on occupational risks is pointing in the same direction. Dr Edward P. Radford, professor of environmental epidemiology at the University of Pittsburgh told the subcommittee that there was now evidence that the risk of cancer induction from radiation might be twice as high as had been estimated as recently as six years ago.

"I believe the whole-body occupational exposure limit should be reduced at least to 500 millirem per year, with careful review of the limits of exposure to individual organs such as the thyroid or bronchial system. This is a matter that is long overdue for change," Dr Radford said.

Although speaking in a personal capacity, Dr Radford's remarks were particularly significant in that he is chairman of the National Academy of Sciences advisory committee on the Biological Effects of Ionising Radiation (BEIR).

The BEIR committee, a respected—if sometimes controversial—source of scientific advice on the dangers of radiation, was initially established in 1970, partly in response to the public debate on the effects of nuclear fallout occurring at that time. Its first report, published in 1972, made little specific reference to occupational exposure limits; and partly in order to remedy this, the committee was asked last year by the Environmental Protection Agency to produce a follow-up report, publication of which is due in September.

The scientific measurements so far are still widely contested, the heat of the debate often appearing in direct proportion to the scientific uncertainties involved. But as Dr Seymour Jablon of the National Academy of Sciences, a fierce critic of some of Dr Mancuso's and Dr Stewart's conclusions said of the Hanford study at the recent AAAS meeting: "Certainly there's something peculiar going on." □

Universities can patent recombinant DNA results

THE RESULTS of university research using recombinant DNA techniques can be patented in the usual way, and do not require any special form of patent agreement, according to Dr Donald Frederickson, Director of the US National Institutes of Health.

The only proviso is that licences can be granted under patents secured on any invention only if the licensee provides an assurance that he or she will comply with the physical and biological containment standards laid down in the NIH guidelines for recombinant DNA research.

Dr Frederickson's decision, which was announced in Washington last week, follows eighteen months of discussion initially sparked off in 1976 by the decisions of Stanford University and the University of California to file a patent application on a process for forming recombinant DNA.

In deciding that no special patent machinery needs to be introduced to cover such research, Dr Frederickson has rejected the suggestions that since the research has potentially wide applications and has been financed largely from public funds, any patents arising from it should become the property of the US government.

This suggestion had received the support both of a number of scientists and other groups involved in the

recombinant DNA debate, and of the Department of Justice. The original version of the bill covering DNA research which Senator Edward Kennedy introduced into Congress last year—and later withdrew—was also based on the concept of government ownership of recombinant DNA inventions, although this was omitted in later versions of the bill.

In announcing his decision, Dr Frederickson said that comments received from individuals consulted by the NIH revealed general support for the existing institutional patent agreements (IPA) between the Depart-

ment of Health Education and Welfare and institutions receiving research grants through the NIH.

An IPA provides the institution carrying out a particular piece of research on department grants or contracts the first option to own all inventions arising from the research, although the government has to be granted a free non-exclusive licence to use the research results.

Dr Frederickson said that there were no compelling reasons to distinguish inventions arising from research involving recombinant DNA from others involving biological substances or processes that have been patented, even when partially or wholly developed with public funds.

"Such inventions include vaccines for rubella and rabies, treatments for herpes virus infections of the eye, treatments of uremia, and prostaglandins—compounds that may have a number of possible medical uses" Dr Frederickson said.

"The argument that commercial development based on patent protection has or will assure maximum benefits of these inventions to the public applies as well to the putative benefits of recombinant DNA inventions".

Under US law an inventor has a one-year period of grace after research results are published in which to file for a patent. **David Dickson**



'We've developed a plasmid that forms the words "patent applied for"'

Spain keeps bargaining for European observatories

THREE nations involved in setting up astronomical observatories on prime Canary Island sites are discovering that the Spanish drive a bullish bargain. Last week scientific representatives of Denmark, Sweden, and the UK, all wishing to establish telescopes on Canary's high and dry peaks, met their Spanish counterparts for 2½ days of hard negotiations. There was substantial progress, but there remain equally substantial points of disagreement.

For the UK's project, the 'Northern Hemisphere Observatory' (NHO), it seems that the principal remaining argument is over the degree of commitment to Spanish astronomers and astronomy in the project. This is, indeed, the central scientific bargain in the whole scheme: that Spain will provide its observing sites, while participating countries will provide time and training facilities at the observatories to help develop Spanish

astronomy. Other bargains, such as the extension of the Canary Islands road network, have already been struck.

The Spanish negotiators are presently arguing that a small amount of time which was to be reserved for international use should revert to bilateral use—divided equally between Spain and another country—and that training facilities for Spanish astronomers should extend to their being invited back to the home institutions of the collaborating groups in an observation. While this is clearly advantageous to the Spanish, it is seen as a difficulty on the other side of the table: observing time reserved for purely international use, is common at large observatories and it is not easy to impose controls, through the agreement, on the fellowship policy of the independent universities and institutions which would compose a collaboration.

Nevertheless these difficulties seem capable of resolution, and UK negotiators are speaking in terms of "a couple of months" before the heads of agreement are signed. Then the whole thing must be turned over to the foreign ministries—when other considerations might come into play quite

outside the realms of astronomy, such as the status of the British colony of Gibraltar: If all went well, there might be agreement in a year. □

Big mirror for UK?

A CHEAP (£1 million) but large mirror blank resting tantalisingly in the works of Owens Illinois, the US optical firm, is giving concern to UK astronomers. Can they buy it for the large telescope planned for the Northern Hemisphere Observatory? The NHO is still a paper project, under negotiation with Spain for a site in the Canary Islands (see above). Finance has been agreed only for moving the cloud-bound 100-in Isaac Newton telescope, leaving the star of the show, a 4.2 metre light collector, unfunded. But with a blank of the right size waiting in Illinois at one-fifth the price a similar blank might fetch in a few years' time, it is tempting to buy it now. But that involves a commitment to the 4.2 metre telescope at some time in the future, and it is not clear that this will be forthcoming from the government. Negotiations continue. □

Bangladesh plans to exploit its natural gas

BANGLADESH has met with little success in exploring for essential minerals. But it has found natural gas in commercially exploitable quantities. Seven gas fields, all in the East, have so far been identified, but few wells have been drilled in these fields and consequently the data required to evaluate the total exploitable quantity of gas are limited. A total of about 9.36×10^{12} standard cubic feet of gas has been estimated in all these reserves, of which the two main fields are Tatas with reserves of about 2.2×10^{12} st. cu. ft and Bakhrabad with about 2.8×10^{12} st. cu. ft. Knowledgeable experts reckon that besides the proven reserves, there may be an additional quantity of about 11.30×10^{12} st. cu. ft, bringing the total to about 20×10^{12} st. cu. ft of gas in fields already discovered.

Tatas is the only field being exploited extensively at present. A 14-inch diameter 55-mile-long pipeline feeds three power stations, one fertiliser

factory, the industrial and domestic consumptions in Dacca and some other areas along its route. The Chhatak cement factory, the Fenchuganj fertiliser plant and the Shahjibazar power station are each supplied from a separate field nearby. On the whole about 100 million st. cu. ft of gas is presently being used in a day by four power stations, two fertiliser plants, 231 industrial units, 934 commercial units and 43,500 domestic consumers.

Bangladesh gas is one of the purest natural gases composed of 95% to 99% methane, very little higher hydrocarbons and inert gases, carbon dioxide, and traces of sulphur. It has a calorific value of 1,050 BTU/st. cu. ft.

Bangladesh at present needs to import annually about 1.40×10^6 tons of crude oil besides coal and other fuels, involving about \$100 million in foreign exchange. Five years hence import requirements are expected to double—so the government has undertaken a programme to maximise the use of

natural gas. Gas is the only source of energy capable of rapidly replacing the bulk of imported fuel.

An interesting prospect would be the replacement of kerosene by methanol produced from Bangladesh natural gas in household applications such as in cooking and lighting. The fact that methanol is toxic and burns with a flame invisible in daytime makes it necessary to investigate the possibility of suitable additives to methanol delivered to households and to design an appropriate system of distribution and handling. Natural gas can also be an excellent raw material for export. It can be transported in natural form by pipelines or in liquid form, by refrigerated truck, railcar or tanker. The liquid is reconverted into gas at the point of delivery without affecting the thermal efficiency. It has, however, been reported that process efficiency, in terms of BTU content, of liquefaction is about 86%, whereas that of conversion into methanol is only 56%.

M. Kabir

USSR Academy plans for 1990

ACCORDING to current Soviet doctrine, the Academy of Sciences has the task of co-ordinating the research efforts of the whole Union and ensuring their application in "accelerating scientific and technical progress". This year's Annual General Meeting of the Academy, falling as it does within the jubilee year of the October Revolution, placed unusually great emphasis on the practical sides of the Academy's work.

In his report, Academician Anatolii Aleksandrov, President of the Academy, noted that a forecast of the development of particular (unspecified) fields of science and technology had been prepared up to 1990. "Important practical recommendations" had been made and were being implemented, aimed at the "socialist integration" of the Comecon block—an ideal which, although somewhat de-emphasised in the current Five Year Plans, still remains an ultimate desideratum.

Turning from planning to more concrete matters, Aleksandrov noted that several new Institutes had been opened, including an applied physics institute (in Gor'kii), an institute for the comprehensive exploitation of mineral resources, and an institute for molecular genetics. These last two subjects are specifically mentioned in the "Fundamental Directions for Science and Technology" of the current Five-Year Plan; indeed, molecular genetics was suddenly upgraded half-way through the previous plan (in May, 1974), an unusual event

which must have proved extremely perturbing to those responsible for the more detailed aspects of State planning.

Other topics mentioned in the "Fundamental Guidelines" include space research and the development of new chemicals. These interests were specially mentioned in the report by Georgii Skryabin, Chief Academic Secretary of the Academy, announcing the winners of the Academy's gold medals. One of the winners could not, however, come forward to receive his medal; cosmonaut Georgii Grechko was unavoidably detained aboard *Salyut-6*.

Marinov recants

STEFAN Marinov, the Bulgarian anti-relativist, caused a considerable stir earlier this year when his book *Eppur si Muove* came out with a preface signed by Academician Andrei D. Sakharov.

According to verbal messages relayed by friends, Sakharov repudiated the preface (see *Nature* 271, 296-297; 1978). Marinov, however, was unwilling to accept these reports, and, leaving his Belgian exile, flew to Moscow as a week-end tourist. He reports the outcome in a letter to *Nature's* correspondent on Soviet affairs:

"Dr Sakharov told me that he had never given any oral message which could be understood as a consent that the foreword written by me and sent about a year ago to him could appear

The highest award of the Academy—the Lomonosov gold medals, had been announced earlier this year; however, a formal announcement was made by Academician Aleksandrov. The Lomonosov medals go, traditionally, to one Soviet and one foreign scientist each year; this time they went to Mikhail Lavrent'ev for work in mathematics and to Linus Pauling for his work in chemistry and biochemistry.

The vast extent of the Academy's work was implicit in all the reports. The Academy now includes more than 250 institutes.

Vera Rich

signed with his name. He had expressed only a general sympathy towards me. He wishes to be no more involved in my fight for the restoration of absolute space-time. I begged him to publish clearly in the press his opinion of my theory; however he refused to do this, because he is dedicating his time to other problems. 'Our ways have crossed once' he said 'and I should prefer that they do not cross another time'.

"I presented to Dr Sakharov my excuses for the highly unpleasant incident of this foreword, and I do this publicly with the present declaration.

"Sakharov's foreword has appeared only in the 1000 copies already published. The foreword will be removed from the 4000 copies which will be printed as 'second edition'."... □

Council of Europe experiments with remote sensing

THE European Joint Committee on Scientific Cooperation, a committee of the Council of Europe, recently conducted what M Andre Boulloche, its chairman, described as an experiment in democracy for Europe: a one-day parliamentary hearing on 'Europe's specific needs in the field of remote sensing' which was held in Toulouse last weekend. The aim of the hearing was to help "parliamentarians in their political decision-making" and to strengthen the democratic process by opening the hearing to the public.

The idea for such a hearing stemmed more from a growing dissatisfaction with the way in which Europe conducted its space programme than from concern over the remote sensing programme in particular. Attempts to implement a coherent European space policy, or even see through a European Space Agency (ESA) project, have often been thwarted by the desire of individual countries to pursue their own interests.

By holding a public hearing, the Council of Europe hoped to initiate interest in space policy as a European affair—both among the parliamentarians of its member states and among the public. Remote sensing was an appropriate topic to spark off that interest: first because it has already suffered from disagreement among ESA members over a future programme, and second because it is likely to have public appeal, being applicable to many practical problems.

The format of the hearing was based on that of Congressional hearings in the United States. The jury, a group of parliamentarians and their scientific advisers, questioned a 'group under challenge'. This group consisted of users of remote sensing from the member countries, and delegates from ESA, the national space agencies, and international organisations. The 'moderator', Mr Van Aal, whose task was to "stimulate a confrontation

Several good cases were put forward for Europe building and operating its own satellite for remote sensing. At present Europe depends mainly on receiving satellite remote sensing data from United States satellites via Earthnet, ESA's European Network to Receive, Reprocess and Distribute Earth Resources Satellite Data. This consists of several receiving stations dotted around Europe, not all of which are yet operational. ESA also has plans to send remote sensing experiments on the first spacelab payload.

But a European satellite would not only relieve Europe of its dependence on the United States, it would also provide the opportunity of designing a satellite geared to Europe's needs and problems. NASA's Landsat satellites, for example, have a ground resolution of 40–80 m, adequate for imaging American farms, but not the much smaller agricultural holdings of Europe. A ground resolution of 10–30 m, however, could be developed for sensing instruments aboard a European satellite.

Another major problem with remote sensing for Europe is that much of the continent is covered with cloud for much of the time. But infrared imaging, microwave and aperture radar synthesis techniques are not affected by cloud cover. The latest Landsat satellite, launched earlier in the month, can take images in the thermal infrared, but microwave and aperture synthesis techniques are not yet fully operational. They could, however, be developed by an ESA member state to be incorporated into a Euro-

pean satellite for launch in the mid 1980s.

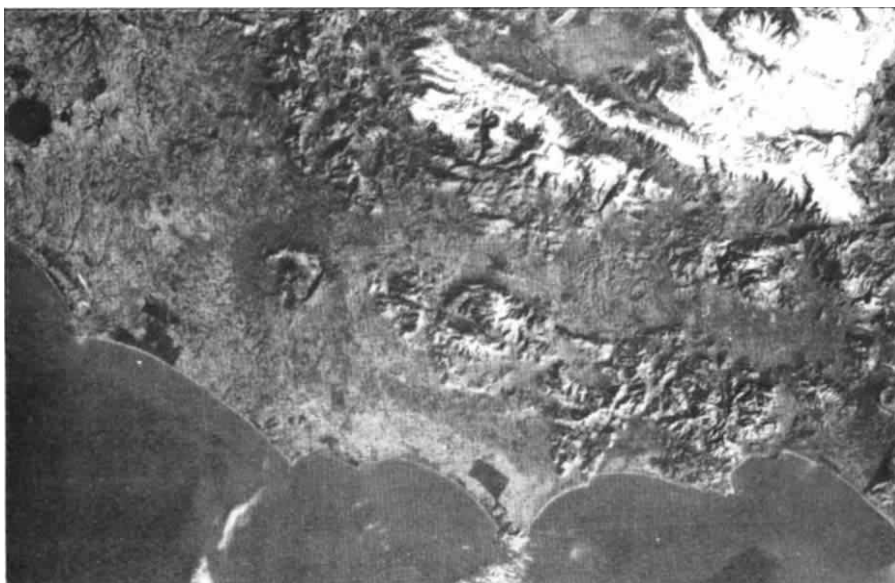
Although the format of the hearing could be compared to that of a United States Congressional hearing, the tone of the debate was very different: it lacked the hard questioning needed to work out a policy. But perhaps that could not be expected from politicians coming from many different countries (except for a notable absentee, the UK) where remote sensing is not a hot political issue. If the hearing did inform and make them aware of an issue of concern to Europe which they would otherwise not have known about, then it could be said to have been a success.

One question, however, raised hackles—on whether France had the capability to develop a satellite incorporating new techniques. M A. Lebeau, director of future programmes at ESA, refused to answer. The French are already building their own remote sensing satellite, SPOT, for launch in 1983. At the end of last year ESA rejected the SPOT proposal. It is similar to NASA's Landsat satellite, and does not use microwave imaging or aperture radar techniques. ESA is currently preparing a programme proposal for a European remote sensing satellite which it hopes to have ready by the end of this year.

The next step is for the Council of Europe's Committee on Science and Technology to draw up a draft recommendation for discussion by its Parliamentary Assembly. M Boulloche summed up the hearing by saying that "European parliamentarians will not leave with any deep rooted conviction about remote sensing. But let's not worry—the United States have held two hearings and still haven't made up their minds". **Judy Redfearn**

Erratum

A typographical error occurred in the article 'Reprocessing nuclear fuel: for . . .' by Professor G. R. Bainbridge on page 2 of this volume. The first paragraph stated that Mr Justice Parker had recommended that BNFL built an oxide-fuel reprocessing plant at Dounreay. This should have read Windscale. We apologise for any confusion this may have caused. between the jury and the 'group under challenge' sat between the two.



The area around Rome photographed by NASA's Landsat satellite

Long live science!

Maxim Gorky (1868-1936), Russian author and friend of Lenin, gave this historic speech on behalf of science at the first public meeting of the "Free Association for the Development and Propagation of the Positive Sciences" in April 1917, under Kerensky's provisional government.

MOST honoured citizens! It will probably seem strange to you that I have decided to bother you with a layman's opinions on science and its significance in the life of reborn Russia, and on the role to be played by science and technology in the new Russian history.

But maybe I shall be able to shake your natural and understandable sceptical attitude to my impudence, if you will permit me to set out briefly my attitude to experimental science and my opinion on the creative work which science can and must accomplish in our spiritually warped country.

Esteemed citizens! I do not know of any forces more creative and more apt for educating social instincts than the forces of art and science. I will say more—being in a known and modest degree a representative of art, I would quite sincerely and consciously put science in first place in the process of man's education.

For art is emotional; all too easily it succumbs to the subjective peculiarities of the creator's psyche; it is too dependent on what is conventionally called mood, and, for these reasons, it is rarely truly free, it rarely transcends the barriers imposed by the powerful influences of individualism, class, and national and racial prejudices.

The experimental sciences, which have developed mightily in the grateful soil of exact observation, are guided by the iron logic of mathematics, completely free from these influences. The spirit of the experimental sciences is truly international and for all humanity. We have the right to speak of Russian, German, or Italian art, but there exists only a single, universal, planetary science, and this gives wings to our thought, raising it up to the limits of universal mysteries, as clues to the tragedy of our existence; this opens up to the world the path to unity, freedom and beauty.

It is not for me to convince you that it is necessary to fill our Russian democracy, which is just at this time

rising again to new life with the exact sciences. K. A. Timiryazev, an outstanding scientist and most upright man throughout his long life stubbornly affirmed 'the future belongs to science and democracy'. This is a great truth. And I am profoundly convinced that unless it goes hand in hand with science, democracy will have no future.

For we Russians, it is especially important to equip ourselves with the exact sciences. For Russians more than for any other nation, it is necessary to inculcate respect for intellect, to develop a love for it, and to become aware of its universal force. It is necessary to understand that intellect is our luminary, that it is magma which can warm us from within, that only on its bright wings can we ascend to a height worthy of man, worthy of his sufferings in the search for truth and his unquenchable thirst for truth.

Russian history has woven for our nation a dense net of conditions which from days of old have inculcated and to this very day continue to inculcate the national masses with a suspicious, even hostile attitude to the creative force of the intellect and the great achievements of science. The ideas of Western European culture were introduced into Russia by the nobility. For the majority of the nation the nobleman was only a landlord, a serf-owner—what good could come from him? In the peasant's opinion, the scientist was a gentleman and not a worker loosing the fetters of the spirit.

Add to this the church-orientated education of the people, which was irreconcilable to beauty and the force of free and fearlessly investigating thought. And further, add to this the monarchical power which both directly and indirectly suppressed any striving for knowledge. In this sum of influences depressing the vitality of Russian man, one may count many more, but this is not the place to discuss this matter. All these hostile influences should have implanted in the Russian a purely organic, instinctively negative attitude to the great quests of science and to the heretical dogmas of scientists.

What way is there out of this joyless picture? There can be only one way out: science, the most active force in the world, must destroy the ancient mistrust rooted in the Russian people.

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Camera Press

It must tear out from the people's soul the scepticism of ignorance, it must set free that soul, precious to all of us, from the fetters of prejudice, and giving it the wings of knowledge, must raise up the Russian people to the highest stage of culture.

The people must learn that now they are living in an atmosphere created for them precisely by science. They must understand that the gentleman gathering flowers in the field is not an idler, but a man who is training an agronomist for the village, that the cotton shirts on their back were made in a mill which could not have been built without a knowledge of mathematics, and that the doctor's medicine is the result of the painstaking work of a scientist. They must know that there exists in the world an intellect which tirelessly and lovingly takes thought for their lives . . .

The atmosphere of science surrounding the city-dweller is even thicker. Here at every step a man may see the triumph of intellect and the enslavement of natural enemies to help him, man. The tramcar and the cinematograph, the automobile and the gramophone, the coat button and the thermometer—all these, useful and ornamental, great and small, were created by science. The process of diffusion of great scientific ideas into the depths of daily life, into dingy Russian existence, is completely incomprehensible to the man in the street, although all his life is penetrated and filled with the ideas of experimental science, crystallised in different forms of practical life.

I understand that telling the man in the street about science's services to him is the task of the populariser and not that of the scientist who is absorbed in his attempt to discover the innermost mystery of existence. But the significance of the popularisation of the exact sciences is enormous and a matter of great responsibility;

This speech was published in the May-June 1917 double issue of the popular science journal Priroda, which reprinted it last month in the special celebration issue, No. 750. It is here translated by Vera Rich.

enormous because it is the only thing which can restore health to the psyche of Russian man, and because it alone is capable of creating an atmosphere of sympathy towards the highest aims of science and to evoke the trust of the masses in the force of the intellect.

And hence it seems to me that the foremost problem for us, as far as cultural significance is concerned, is to create in Russia an organisation which would concentrate in itself the whole work of the intellect aimed at the experimental investigation of the great mysteries of existence. I envisage this organisation in the form of a free association of scientists, an association which, working on a world scale, would be in direct spiritual intercourse with similar associations such as, for example, the British, and would strive, in addition to its direct problems, to create in the world a single, planetary receptacle for science which would be the brain and nervous system of our planet.

It is especially necessary to create such an organisation here in Russia, where intellect still does not enjoy its due respect and where its freedom of action was so cynically restricted by the untalented uncultured yoke of the monarchy.

There is no country where science, the highest expression of the life of the nation, could have existed further in the background, where its free striving was thought more dangerous, and where men of science were treated more abominably, than in Russia under the old regime. We ourselves know how brazenly and rudely the hand of politics touched the pure wings of science. You remember how many strong men from among our scientists were forced to leave their homeland, and how many fine talents perished in it, unable to use their forces. But now before men of science a happy possibility has opened up of freshly organising themselves for their wonderful work, for the limitless widening and deepening of the frontiers of the exact science, for the resurrection of the Russian people from the dead.

Permit me to indulge in fantasy—I do this in the profound belief that there is no fantasy which the human will and intellect cannot transform into reality.

I envisage an establishment which I would call the "science city"—a row of temples where each scientist is a priest independently serving his own god. It is a row of excellently equipped laboratories, clinics, libraries and museums, where from day to day the bright intrepid eyes of the scientist gaze into the darkness of the dread mysteries that surround our planet. Here are the forges and workshops

where the men of the exact sciences, smiths and jewellers, forge and facet the whole experience of the world, transform it into working hypotheses, into weapons for further quests into truth.

In this "science city" the scientist will be surrounded by an atmosphere of freedom and independence, an atmosphere stimulating creativity, and his work will create in the country an atmosphere of love of intellect, and will evoke among people a proud affection for its power and beauty...

I believe that for men of intellect democracy will take on the significance of an exact science. I know that democracy loves exact science. And I will say that within your will lies the spiritual rebirth of Russia. Let there be light on Russia.

In these days, when over our sorrowing suffering country there has blazed up a flame, the dawn of a new life, when the Russian people has come to feel the joy of freedom, in these happy and long to be remembered days, men of intellect, men of science, cannot stand aside from great events.

History will call them to the place which by right belongs to them—in the front ranks of creating a new life. It is they who must lead the country. It is their right to feed the spiritually hungry people from the treasure house of planetary intellect, of world science. . . .

We have destroyed the old structure of life only physically—spiritually it is still around us and even within us. Herculean forces will be needed to cleanse ourselves and the whole country from the grime and rust of the monarchical regime. . . .

We need to learn how to live, to work, to love our labour. We need to

understand that labour is not an imposition on our will, labour is the free expression of the will to live, and in free labour, just as in love, is concealed the most supreme delight. This must be understood, and only exact science can help us to understand, only by filling ourselves with the spirit of the positive science, can we gradually become healed of our grievous defects.

Citizens!

Culture has three foundations: science, and industry. Permit me to remind you of the great work of the Convention Nationale in France from 1791–1793. In these three years, the convention, living in an atmosphere of chaos and terror, under the threat of foreign invasion, extended the three departments founded by Buffon to twelve and established a botanic garden which all Europe might envy, founded a Conservatoire of Art and Trade, and three medical schools. The Convention decided, during a war, to strain every nerve to exempt professors and students from military service.

In inexpressibly difficult conditions, the Convention found it possible to publish for farmers "Counsels for autumn sowing" and at its initiative the scientist Dubanton wrote his classic "Handbook for shepherds". The Convention undertook the draining of the marshes, and the organisation of model farms; in 1793, at the height of the terror, it installed a bust of Descartes, the father of French philosophy, in the Pantheon, published the works of Bacon, sent out scientific expeditions, founded an agronomical bureau; moreover, Tampioni, with the backing of the Convention, struck the first blow for the excavation of Pompeii. . . .

I would recall that the British Association of Scientists (sic) arose in 1810, at a time when England was on the verge of disaster. It is for us, citizens, to organise in our country its best brains, its creative nerve force; we have to create for the development of Russian science those conditions which would give it the possibility of free and infinite development; we have to concern ourselves, in a friendly way, that our scientists may give the country their maximum creativity.

The higher ascends freely investigating science, the wider its horizons, the more abundant the possibilities of the practical application of scientific knowledge to the conditions of life. In nature, as we know, there is nothing more marvellous than the human brain, there is nothing more wonderful than the process of thought, nothing more precious than the results of scientific research.

Long live science! □

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Novosti

Akademgorodok, the science city in Novosibirsk, realised one of Gorky's dreams

correspondence

Low-level radiation

SIR,—Several times in the last two years and particularly during the Windscale hearings it has been suggested that the linear hypothesis for production of cancer or mutations by radiation could not be relied upon at very low levels of dose. It has, of course, for some time been recognised that there is and can be no actual evidence for damage to humans by very low levels of dose since the effects to be expected are so common from other unknown causes that there would be no hope of distinguishing a few extra cases resulting from low-level doses even if these were to affect large populations. There could thus be a threshold dose below which the effects were zero. This seems unlikely on theoretical grounds and it is obviously wise to play safe by assuming that there is no such threshold.

The suggestion with which I am concerned, however, is that very low levels of dose might have effects which are actually larger than those to be expected from the linear hypothesis. This seems to be highly improbable for the following reasons:

It is generally agreed from the near linearity with dose of the incidence of malignant disease due to higher levels of radiation that the primary effect of radiation is to produce a permanent change in a single cell. The cell is thereby either caused later on to undergo uncontrolled proliferation or alternatively is left in a state in which it can be induced to do so by some common external cause occurring later in the life of the individual concerned. At the dose rates with which I am concerned (below 100 mrems) any non-linear theory would require some very improbable assumptions.

I will consider first the alpha-emitting radioactive materials, which are most dangerous and which are perhaps causing the greatest concern owing to their long half-lives. If a single alpha particle dissipated its energy in a single cell (mass perhaps 3×10^{-6} of a gramme) it would give a radiation dose to that cell of about 25 rems (assuming a quality factor of 10). Since the range of one of the alpha particles would be a little more than the diameter of a typical cell one can assume that in a large proportion of cases the alpha energy will be divided between 2 or 3 cells so that the average dose to each cell affected would be in the region of 10 rems.

If then a person is receiving a local dose of 100 mrems per year due to alpha-emitters, an individual cell can expect to be struck by an alpha particle once in 100 years. The number of cells struck once is of course proportional to dose, and this implies exceedingly strongly that any effects in this dose range must vary linearly with dose. If smaller dose rates still had a larger than proportional effect, a second event, happening decades later, would have to be able to cure the effect of an early event. At high dose dose rates this could be true since there is a fair chance that

a second alpha particle would kill a previously affected cell outright, but it does not seem sensible to suppose that individual cells can be cured of whatever damage has been incurred by further radiation damage, many years later, either in the cell itself let alone in one of the surrounding cells.

The same argument applies to beta and gamma radiation although here for a given number of mrems per year the number of cells receiving a significant energy input is perhaps 10 or 20 times larger. In this case, however, since everyone in this country has a background dose of 100 mrems or more, what we would have to believe is that although this first dose between perhaps 100 to 200 mrems has an unobservably small effect, an additional dose of a few mrems anywhere in this range may have a disproportionately serious effect.

Your faithfully

JOHN FREMLIN

Department of Physics
University of Birmingham

Pit latrines

SIR,—In many places the best way of providing sanitation for the millions in developing countries who live in rural areas and makeshift city accommodation is still the pit latrine. At Loughborough a team is investigating the ubiquitous privy along with other forms of unsewered sanitation. Unfortunately there are precious few facts and figures to work on.

In the days of the Empire, latrine programmes often fell to the lot of government agents, district commissioners and other administrators who were renowned for scrupulous note-taking. I wonder, Sir, whether any of your readers have retained notes or have a clear memory of the operation of pit latrines. If so, will they please get in touch with me, especially if they have information about such things as the life of the pit, groundwater pollution, successful modification to prevent nuisance, and use as fertiliser of the mature contents of pits.

Your faithfully

JOHN PICKFORD

University of Technology
Loughborough

Nature and nurture

SIR,—In recent years a great deal of heat and little if any light has been generated by discussions of the inheritability or otherwise of 'intelligence' (whatever this term may mean), especially as applied to supposedly genetically isolated ethnic groupings. To my knowledge, no attempt has been made to examine whether there are any simpler or more readily defined characteristics than 'intelligence' which could illuminate whether there was any scientific point whatever in such investigations. This note attempts to help in this respect.

Few would deny that physical attributes are less complex than mental

attributes. Furthermore, there exists a hierarchy of complexity in human physical attributes. Some (for example, colour of eyes or hair, colour blindness, and haemophilia) are easily defined, readily measured, constant throughout life, and it seems to be reasonable to classify them as 'simple'. Next one might take measures like bodyweight or height or size of biceps or girth at various ages, characteristics that might be called 'intermediate' in this classification. The highest forms of athletic skills (pole vaulting, running, pentathlon etc) are however without doubt highly complex.

There is little doubt that the 'simple' attributes are genetically determined, as is established by massive investigations, nor is there much debate that both genetic and environmental factors (such as nutrition) are important for the 'intermediate' characteristics.

As regards the highly complex athletic skills, they are measured in various forms of international competitions, notably the Olympic Games. Due to the political circumstances of the post World War Two period, an illuminating amount of information has become available.

Before 1945, the genetic differences between the areas that became East and West Germany respectively were quite modest, certainly by comparison with the differences between major ethnic groupings. After 1945, there was first a mass movement of Germans from further east, largely to West Germany, and then, until 1961, a substantial movement of people from East Germany into West Germany. The first of these would have diminished yet further the genetic difference between the two, as would the second (which, some would say, had impoverished the genetic pool of East Germany).

Since the DDR (East Germany) has participated in international sporting contests, the outstanding performance of their athletes has excited widespread comment, particularly when the modest size of the population of the DDR is remembered. There is thus little doubt that the great support given to sport (and athletics in particular) by the DDR has enabled the country to put up a remarkable performance relative to the bigger and richer West Germany. Since this success of the DDR can hardly be ascribed to genetic differences vis a vis West Germany, it follows unambiguously that environment must have a commanding part to play in establishing complex human physical achievements, in spite of the undoubtedly genetic determination of simple physical characteristics, such as eye colour.

With such evidence plainly in front of us, what scientific sense can be assigned to attempts to establish the genetic basis of such incredibly complex attributes as 'intelligence'?

Yours faithfully

HERMANN BONDI

Department of Energy
London

news and views

Too many electron neutrinos?

from D. J. Miller

A CURIOUS and completely unexpected effect has been found in the 'beam dump' experiment at CERN. An experiment with a modified version of the CERN neutrino beam has shown that the ratio of the number of neutrino-induced events producing an electron to those producing a muon was seven times greater than expected from contemporary theories.

The normal neutrino beam at CERN is derived from a mixed beam of π and K mesons which is produced when protons from the super proton synchrotron (the SPS) collide with a small metal target. The pions and kaons have lifetimes of around 10^{-7} s. In a tunnel some hundreds of metres long a large fraction of them decays, giving a mixture of neutrinos and charged particles, mostly muons. A thick shield of steel, concrete and earth stops the charged particles, but the neutrinos pass through the shield and into the detectors. For the beam dump experiment a thick metal plug was placed immediately after the small target in the proton beam. The bulk of the pions and kaons would interact in the plug and never enter the decay tunnel, so it was expected that the number of neutrino interactions seen in the detectors would be substantially reduced.

But why do a neutrino experiment with such a spoiled beam? This special short run had been asked for by a CERN-Dortmund-Heidelberg-Saclay (CDHS) collaboration who were puzzled by the properties of neutrino-induced events with three final-state muons which had been observed both in their massive steel detector and at Fermilab in the USA (see *News and Views*, 269, 286; 1977). Perhaps these trimuon events were caused by a new kind of neutrino which might be produced by the decay of a short-lived particle. If this particle had a lifetime of 10^{-11} or less then it would be expected to decay rather than to interact, even if it were passing through a

dense metal target or plug. So the beam-dump experiment should see just as many events due to the new kind of neutrino (if it existed), but far fewer events caused by the neutrinos from pion or kaon decay.

Oddly enough, the excitement so far about the beam-dump results does not concern trimuon events at all. As usual, the neutrino beam was used simultaneously by CDHS and by two big bubble chambers, Gargamelle and BEBC. About 70,000 pictures were taken in each chamber, and scanned within about 6 weeks to find all the neutrino events. No one expected to see useful numbers of trimuon events in these scans—the bubble chambers are not massive enough. Events were simply classified as having an associated muon, an associated electron, or no associated lepton (electron or muon). An event with a muon was assumed to be due to a charged current (CC) interaction of a muon neutrino, an event with an electron was assumed to be due to a CC interaction of an electron neutrino, and events without a lepton were taken to be neutral current (NC) neutrino interactions.

The ratio of electron events to muon events in BEBC was 15 to 34 and in Gargamelle it was 9 to 18. But the predicted number of electron events, given this total of 52 muon events, was only 3.5. This prediction is based on the known production and interaction rates of pions and kaons, together with various other neutrino-producing processes such as hyperon decay. The CDHS detector has difficulty in separating electron CC events from NC events, but this collaboration also reports an excess of events without a final-state muon.

The obvious kind of explanation for a greater number of electron events than expected is to assume that there is indeed a short-lived neutrino parent which becomes proportionately more important when the pions and kaons have been absorbed in the plug of the beam-dump. This parent must decay almost as frequently to give an electron neutrino as it does to give a muon neutrino. But results at e^+e^-

machines and in other neutrino experiments have established the existence of just such a particle—the D meson, a charmed particle with a mass of 1.85 GeV/ c^2 (see *News and Views* 262, 537; 1976). D mesons are known to decay about 10% of the time to give an electron and an electron neutrino in the final state, and about 10% of the time with a muon and a muon neutrino. The lifetime is around 10^{-13} s so there is very little chance that a D will interact before it decays. Unfortunately, to account for the observed rate of electron events in the beam dump experiment, the rate of production of D mesons must be about 1 in 200 proton interactions. A number of different experiments over the past few years have placed upper limits on the possible rate of D production at less than 1 in 20,000 proton interactions. So the obvious explanation seems to be ruled out, and new theoretical ideas may be needed to explain the results. Perhaps the τ lepton, which has been observed in e^+e^- collisions, has a neutrino related to it which causes these events and the trimuons too. Maybe the neutrinos from D meson decays interact more strongly than the neutrinos from pion and kaon decays.

Further results from the beam-dump experiment are eagerly awaited. Already it seems that the overall event rate is a little higher than expected and there may be more NC events than in normal neutrino experiments. It is possible that a second beam-dump run will be done during 1978, with the target at the end of the decay tunnel rather than at the beginning of it. This would give a considerable increase of neutrino flux through the detectors, but high radiation levels in the tunnel make it hard to see how the equipment can be installed for such a move. Meanwhile, normal neutrino experiments are continuing and there are tantalising rumours of new results from these which could be as significant as the beam dump data.

Results are being submitted to *Physics Letters* by the CERN-Universities collaborations who analysed the bubble-chamber film. □

D. J. Miller is in the Department of Physics and Astronomy at University College, London.

Influence of bromodeoxyuridine on viral and cellular gene expression

from Robin Weiss

ON page 284 of this issue of *Nature* Biquard and Aupoix report the induction of a 'tumour-specific surface antigen' (TSSA), as well as a C-type virus antigen (p27), in normal avian fibroblasts by treating the cells in culture for 15 days with 5-bromodeoxyuridine (BUdR). The 'TSSA' cross reacts with an antigen expressed on rat tumour cells (XC cells) transformed by Rous sarcoma virus (RSV). Biquard and Aupoix think the antigen represents either a tumour-specific antigen related to a *src* (transforming) gene product of RSV or an embryonic antigen. Cross-reacting embryonic antigens expressed on both avian and mammalian cells transformed by RSV have been reported previously by Kurth and Bauer (*Virology* **56**, 496; 1973). Normal avian cells contain DNA sequences related to *src* sequences which are located in a different chromosome from endogenous virus sequences (Padgett, Stubblefield & Varmus *Cell* **10**, 649; 1977). It is a moot point whether proteins putatively coded by endogenous *src* genes should be regarded as embryonic or tumour-specific antigens.

Despite an increase in agglutinability by concanavalin A, the BUdR treated chick cells are morphologically non-transformed. Indeed, they appear to be flatter and better monolayered than untreated cells, a feature which has also been observed with neuroblastoma cells (Schubert & Jacob *Proc. natn. Acad. Sci. U.S.A.* **67**, 247; 1970) and melanoma cells (Silagi & Bruce *Proc. natn. Acad. Sci. U.S.A.* **66**, 72; 1970). The relationship between BUdR-induced 'TSSA' expression and neoplastic transformation therefore remains obscure.

The effect of halogenated pyrimidines on gene expression in cell culture has been studied in many laboratories. Low concentrations of BUdR were shown to suppress reversibly the expression of differentiation in myoblasts, chondroblasts and melanoblasts (Bischoff & Holtzer *J. Cell Biol.* **44**, 134; 1970; Lasher & Cahn *Devl Biol.* **19**, 415; 1969; Coleman *et al. Devl Biol.* **19**, 527; 1969; Rutter *et al. A. Rev. Biochem.*, **42**, 601; 1972). BUdR and iododeoxyuridine (IUd) were also found to be potent inducers of endogenous murine and avian C-type viruses (Lowy *et al. Science*, **174**, 155; 1971; Aaronson *et al. Science*, **174**, 157; 1971; Robinson *et al. Virology*, **69**, 63; 1976). Is there some close relationship between viral gene induction and suppression of differentiation? Apparently not, for the

effects of the halogenated pyrimidines are mediated through quite distinct pathways. Meuth and Green (*Cell*, **2**, 109; 1974) have shown that BUdR inhibits cellular ribonucleotide reductase activity and can kill cells by starving them for deoxycytidine nucleotides. The addition of deoxycytidine in the medium prevents this toxic effect, although the BUdR becomes extensively incorporated into DNA in place of thymidine. It seems that the suppression of specialised differentiated functions in cultured myoblasts (Rogers *et al. Nature*, **256**, 439; 1975) and melanoma cells (Davidson & Kaufman *Cell*, **12**, 923; 1977) induced by BUdR treatment is not observed when deoxycytidine is also administered. Thus the incorporation of BUdR into DNA is not the cause of the changes in specialised gene expression. As Schubert and Jacob first pointed out,

The age of Aquarius?

Barry Robson and Malcolm N. Jones

THEORETICAL calculation of the conformational behaviour of biological molecules has raised our level of understanding about basic life processes and left it high but dry. Theoretical calculations have run aground because they neglect the water, or treat it in a very simple way, and experimentalists have not been able to come to their aid because experimental observations on dilute solutions provide only a relatively crude macroscopic view of water of hydration. What is lacking is a detailed microscopic insight into the interaction of water with biological solutes, and many theoretical and experimental workers have expressed the view that exact calculation is beyond the present capabilities of man and computer. Hagler and Moulton (this issue of *Nature*, page 222) and Rossley and Rahman (preliminary report in *Models for Protein Dynamics*, CECAM Workshop, Orsay, 1976) have shown that this view is not justified.

The techniques used are Monte Carlo (Hagler and Moulton) and Molecular Dynamics (Rossley and Rahman). The former exploits an efficient technique for the random sampling of water configurations round solute molecules, the sampling continuing until the average properties of the system have converged. The latter follows the development of the system with time by applying Newton's laws

of motion to the component atoms. One problem is that it is only possible to simulate the behaviour of a limited number of water molecules, although this limited number may be several hundred. Without precautions this could be dangerous, because the system would then represent a droplet of solution and there could be serious problems arising from the special behaviour of solvent at the water-vacuum interface. To get around this problem, both groups apply 'periodic boundary conditions', which means that each face of a basic volume of solution containing one solute molecule is in contact with an exact replica of the basic volume, and these volumes are stacked against each other *ad infinitum* to fill space. This is rather like the repeating unit cell of a crystal, except that the system is in simulated motion. Hence a water molecule leaving at a top right corner of the unit cell re-enters at the opposite lower left corner.

Rossley and Rahman include sufficient water molecules in a cubic unit cell and use a cube of sufficient size so that the periodic boundary conditions do not lead to anomalous effects due to long-range order, that is, the system is supposed to approximate infinite dilution. They thus apply their

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CHIRON, the mythological quadruped, half man, half horse, and a descendant of the gods Saturn and Uranus, is the name given by Charles T. Kowal to the fast moving object he discovered on 1 November, 1977 (see *Nature, News and Views* **270**, 385; 1977). In common with many other 'new' discoveries in the Solar System Chiron had been observed before. Knowing roughly where it was helped enormously. Careful sifting of the astronomical photographic plate collections have brought to light over 30 images, the oldest dating back to 1895. These images have been used to obtain an accurate orbit, which, unlike the preliminary orbit given in the reference above, now has a perihelion distance of 8.51 AU, a whole astronomical unit within the orbit of Saturn. Chiron passed perihelion in 1895 and 1945 and will pass again in February 1996. At perihelion it has a photographic magnitude of 14.5, 40 times brighter than it is at present. The orbit is inclined at 6.923° to the ecliptic plane and at aphelion Chiron is 18.9 AU from the Sun, only about two astronomical units from Uranus.

There has been some discussion as to whether Chiron is an asteroid or a comet, the majority opinion favouring the former. Most comets far from the Sun show little or no nebulosity and are virtually indistinguishable from minor planets on an observational basis. However, Chiron's estimated radius lying as it does between 50 and 320 km makes it larger than all known comets but well within the range of observed asteroids.

Assuming it is an asteroid still leaves us with a fascinating problem—where did it come from? There are two simple possibilities, first it originated near its present orbit and is a prominent member of an asteroid belt that has always existed between Saturn and Uranus. There is a discontinuity in planetary masses here just as there is between the planets Mars and Jupiter which bound the inner asteroid belt. In the second theory asteroids have at some time been perturbed from the inner belt into orbits between Jupiter and Saturn and Saturn and Uranus. This latter theory is favoured by Robert C. Smith of the Astronomy Centre at the University of Sussex and is dis-

Chiron's origin

from David W. Hughes

cussed by him on page 229 of this issue of *Nature*. Smith follows R. B. Hunter's hypothesis that the original spatial distribution of asteroids was roughly Gaussian, the maximum number having semi-major axes of about 3.1 AU spacial density values of half this maximum value occurring at around 3.5 and 2.8 AU (see *Mon. Not. R. Astr. Soc.* **136**, 267; 1967). Smith calculates that 330 of the original assumed total of nearly 2,000 would have had mean solar distances between 3.7 and 5.2 AU, this latter value being the semi-major axis of Jupiter's orbit. There are only 50 in this range at present. He concludes that the missing 280 asteroids have been transported outwards by Jovian gravitational perturbations.

The sphere of influence of a planet is defined as the volume around the planet in which the planet's gravitational field has more effect on the orbit of a satellite or passing space probe, asteroid or comet than the gravitational field of the Sun. Inside this sphere the Sun is just regarded as a perturbing influence. Jupiter's sphere has a radius of 0.32 AU, Saturn's is 0.36 AU. Interestingly the ratio between the radius of the Jovian sphere of influence and the distance between the orbits of Jupiter and Mars is nearly the same as the ratio between the radius of Saturn's sphere of influence and the Jupiter-Saturn orbital distance. So Saturn will have a similar influence on the asteroid belt between Jupiter and Saturn as Jupiter has on the belt between itself and Mars. The end product of these gravitational perturbations will be a series of asteroid belts with relative populations in the ratio 1720:240:40 these belts occupying the regions between the orbits of Mars and Jupiter, Jupiter and Saturn and Saturn and Uranus.

As the observed brightness of asteroids is roughly proportional to the inverse fourth power of their heliocentric distance the magnitude

of the brighter members of each group will be approximately 10, 14 and 18.

The faintness of the trans-Saturnian group coupled with their low orbital angular velocities makes them extremely difficult to detect, a fact which helps to explain why Chiron is the only one to come to light so far.

All asteroids with semi-major axis greater than 3.9 AU will have their orbits greatly perturbed by the attraction of Jupiter. According to Hunter some of these asteroids can suffer temporary capture by Jupiter. Most of these will eventually be dispersed into heliocentric orbits well outside and inside that of Jupiter's. Occasionally a capture by Jupiter could become permanent due to successive solar perturbations decreasing the semi-major axis of the captured asteroid's orbit around the planet until this orbit became stable. The eight outermost satellites of Jupiter, together with Phoebe, the outermost satellite of Saturn seem to be captured asteroids. Smith points out that numbers of captured asteroids orbiting the major planets give a crude indication of the number of asteroids available for capture in the asteroid belt just inside the planets orbit.

So the asteroids according to the work of Smith and Hunter are not just confined to the regions of the Solar System interior to Jupiter's orbit. There are asteroids throughout the Solar System and individual asteroids can be quite easily moved from orbit to orbit by gravitational perturbation. They can also spend some of their life orbiting the major planets and a percentage of the asteroids that do this end up as permanently captured satellites.

A preliminary report by Brian G. Marsden of the Smithsonian Astrophysical Observatory of a detailed celestial mechanical analysis of the effect of Jupiter and Saturn on the orbit of Chiron, indicates that it passed within 0.1 AU of Saturn in 1664 and within 0.6 AU of Jupiter sometime in the latter part of the second millennium BC.

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simulation to a system for which infinite dilution is meaningful, and have chosen as solute N-acetyl alanyl N'-methylamide ($\text{CH}_3\text{CO.NH.CH.CH}_3\text{CO.NH.CH}_3$) which may be considered as a model of the smallest possible 'protein'. On the other hand, Hagler and Moulton exploit the resemblance of

the model to the structure of a crystal, and thus chose two hydrated crystal systems. These are the cyclic peptide cyclo-(L-Ala-L-Pro-D-Phe)₂ and the enzymically active protein lysozyme. Their study thus has the advantage of involving more typical biological peptide molecules, and further, since the

positions of the more ordered water molecules in these crystals is known by X-ray crystallographic analysis, it can be checked against experiment.

Briefly, the studies of both groups support the experimental evidence that there are several water molecules strongly bound to the peptide so that

their motion is restricted compared to their behaviour in pure water. Emphasis is placed on special behaviour of water in the innermost shell (up to 3–4 Å from solute atoms). There is hydrogen bonding between solute and solvent, and significant changes in the behaviour round nonpolar groups. However, the water closest to the solute is still labile compared with earlier 'ice-like' or 'clathrate lattice' models of water structure around molecules. Hagler and Moulton summarise this more realistic flexible picture of solute structure by plotting the root-mean-square deviation of water molecules from a mean position as some function of 'intimacy' of contact with lysozyme. However, even this provides only a sketch of the true situation because there is considerable variation from water molecule to water molecule. In this respect, the most important conclusion to date is probably that reasonably good predictions are achieved, depending to some degree on the potential energy expressions and parameters used, of the positions and freedom of motion of individual water molecules in the cyclic peptide and lysozyme crystals studied by Hagler and Moulton.

The molecular dynamic simulation of Rossley and Rahman has the advantage, however, of including conformational motions within the solute molecule. Although these are of less interest in the hydrated crystal systems, they are by no means irrelevant. Unfortunately, it may well be that neglect of quantised vibrational behaviour is more serious in the intramolecular case and it is likely that the motion of the water in the Rossley-Rahman simulation, rather than the solute dynamics, will attract the greatest interest.

A particularly interesting facet of the Hagler-Moulton study is the prediction of water channels of low energy water molecules which appear to 'glue' protein molecules together across distances of 6–7 Å. The authors suggest that this may provide 'long range forces' in biological systems, and there is certainly experimental data which suggests such forces (for example, Gingell *et al.* *Nature*, **268**, 767; 1977). However, it is not clear that the phenomenon predicted is of sufficiently 'long range' to explain all data of this type.

Hagler and Moulton also suggest that their techniques could be extended to simulations of unfolded proteins in aqueous solution and hence to the energetics of hydration on unfolding. This may turn out to be a difficult problem especially when it is realised that even native protein molecules in solution undergo sizeable individual fluctuations in conformations involving

energies of the order of 150 kJ mol⁻¹ and volume changes of the order of 30 cm³ mol⁻¹ (Cooper *Proc. natn. Acad. Sci. U.S.A.* **73**, 2740; 1976). However, if exact calculations of the changes in, for example, heat capacity on unfolding were possible, comparison with the excellent experimental results available should prove interesting (Privalov & Krechinashvili *J. molec. Biol.* **86**, 665; 1974). Such a comparison would be of very general interest since the changes in heat capacity on unfolding are believed to arise primarily from the exposure of hydrophobic residues to water and constitute a classical example of hydrophobic bonding. Furthermore, the efficiency of the theoretical method would be put to a stringent test and success could help to dispel worries of some experimentalists concerned about the covert assumptions implicit in such complex calculations.

To conclude, one is left with the feeling that naive models of water structure around biological molecules have not been particularly useful, and that computer descriptions of water behaviour are necessary in order to model the irreducible complexity of such systems. There are, however, some hints in the present studies which may lead to simplification in future work. In particular, the observation that water not too far out from the solute resembles pure water in its properties opens the possibility of neglecting these molecules, and replacing their effect by a 'continuum reaction field'. In other words, it may be possible to carry out a hybrid computation in which intimate solute-solvent contacts are treated exactly, while longer range effects are treated by classical macroscopic models.

Predaceous insects in aphids' clothing

from Robert M. May

IN the outer office of the Librarian at the Princeton Theological Seminary is a model of a small woolly sheep. A second glance shows the wooden face to be oddly lupine. On close examination, the woolly coat can be removed to reveal . . . a wolf in sheep's clothing. A remarkable example of a predaceous insect larva that follows such a wolf-in-sheep's clothing strategy with almost Aesopian exactitude has recently been documented by Eisner *et al.* (*Science* **199**, 790; 1978).

One of the most familiar symbiotic associations is between ants and aphids. In return for the sweet 'honeydew' the

aphids excrete and which the ants milk by stroking the aphids with their antennae, pastoral ants protect the aphid herd from predators (Way *A. Rev. Entomol.* **8**, 307; 1963).

Enter now the larva of the green lacewing (*Chrysopa slossonae*) which lives among colonies of the woolly alder aphid (*Procipilus tessellatus*) in New York State. It feeds on these aphids by piercing them with hollow mandibles, sucking out their contents, and discarding the shrivelled remains. The association is apparently obligate on the part of the larva, as Eisner *et al.* found them with no other aphids. The aphids are clothed in dense woolly tufts, comprised of thin filaments of a white wax that is exuded from integumental glandular cells.

The extraordinary thing about the chrysopid larvae is that they cover themselves with woolly wax plucked from the aphids. This wool is anchored to long hooked bristles on the back of the larvae; these bristles show up clearly in scanning electron micrographs. As Eisner *et al.* clearly demonstrated, since the larvae also match the aphids' size, these chrysopids-in-aphids-clothing are very hard to distinguish. The authors claim 'The mimicry is astonishing, and only after experience and at close range can the human eye come to discern them.'

When Eisner *et al.* placed freshly denuded larvae among aphids of several colonies, in all but 4 of 27 cases the patrolling ants removed the larvae, either by plucking them off the branch and dropping them to the ground (16 cases), or by carrying them all the way to the ground (7 cases). Of 23 'control' larvae introduced into colonies wearing their sheep's clothing, all eventually settled in among the aphids (8 times after tentative poking by ants, 15 times after no more than a cursory inspection).

A second series of experiments compared the choices made by larvae when introduced to (antless) colonies with or without their deceptive clothing, and when sated or starved. Fed and clothed larvae loafed; hungry and clothed ones ate; sated and denuded ones set about clothing themselves. The interesting case was the hungry and denuded larvae. These hapless beasts divided their time roughly evenly between feeding and applying the protective camouflage.

This is a beautiful and detailed example of coevolution involving three species: ants, aphids and neuropterans. Many other tales can be told of complex relations both among the social insects, and between social insects and other arthropods. An introduction to this literature is in chapters 19 and 20 of E. O. Wilson's book on *The Insect*

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Societies (Harvard, 1971). Writing of social parasitism within the ants, Wilson surveys a range of behaviour including that of 'slavemaker' ants who have become dependent on workers of other species, and 'inquilinism' in which the parasitic species spends its entire life cycle in the nests of the host species. He notes that 'a rich variety of new parasitic species, representing almost every conceivable evolutionary stage, have been added since the time of Wheeler's classic synthesis in 1910. They continue to be discovered at such a consistently high rate as to suggest that, at this moment, only a small fraction of the total world fauna of social parasites is known'.

A very large number of other animal species, particularly other arthropods, exploit the colonies of social insects. Setting aside casual relationships, and considering only those symbionts which live on or among their hosts, Wilson tabulates some of the 17 orders, 120 families, and hundreds of genera thus involved. For symbionts which spend much of the time with social insects in the open, an evolutionary premium is placed on visual resemblance to the hosts or their herds. The chrysopid larvae discussed above provide a fine illustration. Among the many other examples are various species of staphylinid beetles that march with army ants. Not only do these beetles resemble ants in the overall slender body form, in colour, and in the sculpture of the body surface, but ant-like petioles ('wasp-waists' between thorax and abdomen) have evolved in no less than seven distinct ways among this family of beetles (Seevers *Fieldiana Zool.* 47, 137; 1965). For symbionts that spend their lives underground, there is less tendency toward morphological mimicry, and more toward cracking the chemical and behavioural codes of communication among the hosts. An example of this kind again comes from the Staphylinidae, where larvae of *Antemeles pubicollis* masquerade as ant larvae (of the genus *Formica*), apparently by producing a passable imitation of the correct pheromone plus a few crude signalling movements; thus ensconced, the beetle larvae both eat ant larvae and successfully solicit food from the *Formica* workers (Holl Dobler *Sci. Am.* 224(3), 86; 1971).

An interesting question discussed by Wilson is why do social wasps and bees have fewer guests? Not only are fewer species associated with the nests of social bees and wasps than with ants and termites, but their adaptations for symbiotic life are generally far less pronounced. Wilson notes that bees and wasps tend to construct 'compact, tightly sealed nests in arboreal locations'. In addition, bees and wasps

are tidier housekeepers, thus providing fewer initial opportunities for scavengers; bees tend to produce small amounts of refuse (because pollen and nectar are relatively concentrated food sources), and in many species the workers collect detritus and dump it out of the entrance. This contrasts with the many chambers and galleries, created in a matrix of soil or rotting vegetable matter, that characterise the nests of most social ants and termites. Wilson thus suggests that in the chancy game of evolution, the bees and wasps may simply have presented fewer opportunities for coevolutionary adaptations. □

Greener grass?

from Peter D. Moore

GRASSLANDS are an important public amenity in Britain, yet only a minute proportion of what the public spends on their establishment and maintenance is assigned to research. Such is the message of a review entitled *Amenity grasslands—the needs for research*, compiled by a committee of the Natural Environmental Research Council under the chairmanship of A. D. Bradshaw with the full time assistance of M. J. Liddle. The review began in 1973 and was published in December 1977 in response to a request concerning future research needs from the Department of the Environment.

The assembled figures emphasise the extent and the use made of amenity grassland in Britain. ('Amenity grassland' is defined as "all grass with recreational, functional or aesthetic value and of which agricultural productivity is not the primary aim".) The total area of amenity grassland is estimated at 8,500 km² (1.5 times the area of the county of Norfolk or 3.7% of the land surface of the UK). Of this, 4,100 km² is semi-natural (including rural road verges, nature reserves, picnic areas, camp sites and so on), 1,100 km² is intensively managed (including bowling greens, football pitches, horse-race tracks and school playing fields), 2,700 km² is man-made and trampled (including urban parks, domestic lawns and urban road verges) and 600 km² is untrampled (including cemeteries, airfields and motorway and railway embankments). The area may be increasing by about 1% per annum.

The value of this grassland to the public can be judged by the fact that up to 40 million people visit an urban park every week; 10 million people

visit the countryside of Britain on fine weekends and grassland ranks among the most popular habitats; 25 million people may be expected to attend a football match during a season. Add to this the number of people who spend their weekends either reclining on or mowing their lawns and it becomes evident that amenity grassland plays a regular part in the leisure activity of almost every inhabitant of Britain.

The cost of maintaining this grassland is borne largely by government, that is, the taxpayer. Government is responsible for £77 million a year in maintenance costs out of a total of £137 million. Urban parks account for £33 million and domestic lawns for £24 million. Evidently the British public pays, either directly or indirectly, for its love of verdure. Mowing accounts for 59% of the expense. It is followed by fertiliser application (14%) and weed control (5%). The most expensive turf in the country appears to be greyhound racing tracks (costing £12,050 per ha per annum to maintain), followed by bowling greens (£7,238) and cricket squares (£2,242). Nature reserves are easily the best buy at 24p.

Sales of amenity grass seed ran to £6 million in 1974. The total weight of seed sold has been rising at an annual rate of 5% and prices have been escalating recently. It is salutary to note that only 4% of the seed sold in 1974 was home grown. There must surely be room for British business expansion in this lucrative market.

The financial investment in scientific research within this multi-million pound industry is surprisingly small. Apart from University Grants Council subsidised research within the universities, only £163,000 was invested in grassland research in 1974/75 and of this only £34,000 was directed towards amenity grassland. It is certainly fair to say that this represents a substantial underinvestment when compared with running costs and with the profits associated with certain types of amenity grassland, such as in the sporting industry and in tourism in its widest sense.

The core of the NERC report deals with the question of whether there are opportunities for worthwhile research which would benefit the amenity grassland industry. Many fundamental questions could, the report claims, be answered given the appropriate research. How can turf be established without expensive top soil layers? How do seedlings of different species and varieties respond to environmental variables and to the presence of one another in their germination and establishment phases? The role of competition between grass species and between amenity grasses and weed species is stressed as a vital one in the

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determination of sward composition. Rather surprisingly, no mention is made of the importance of chemical interactions. This is an area of basic ecological research which may eventually explain many species inter-relationships in grassland and which could be profoundly important in grassland management.

Are legumes in turf more effective in maintaining a nitrate supply in the soil than the application of nitrogenous fertilisers? What are the long term effects of various mowing and fertiliser regimes? Such a question could be answered by detailed studies of swards with recorded management details. The development of new cultivars continues as a result of the work of such institutions as the Welsh Plant Breeding Station at Aberystwyth, but there is a need for better evaluation of cultivars under different soil, climatic and management regimes. The expense of mowing could be alleviated by the development of slow-growing cultivars (a line of research currently being pursued at Sheffield University). If mowing techniques could be made just 5% more efficient, the resultant saving would amount to about £4 million a year.

These and many other possible research developments are proposed in the NERC review. In view of the low current investment in such research, only 0.1% of the annual maintenance costs of amenity grassland, there does seem to be a sound economic argument for the expansion of ecological research in this area. □

Plate tectonics in the Arabian Pan-African?

from G. C. Brown and J. V. Hepworth

DURING the past 15–20 years, students of African geology have recognised three important ancient cratonic shield areas in West and South Africa, containing Archaean and early Proterozoic rocks, separated by younger, later Proterozoic 'mobile belts'. Pan-African ages (500–650 Ma), extended back to 1,100 Ma, characterise younger rocks of the latter group and such ages are recorded extensively in Arabia. Indeed, the so-called Arabian–Nubian shield is made of calc-alkaline igneous and thick clastic sedimentary sequences which have suffered greenschist metamorphism and may be an extension of the N–S trending Mozambique belt of East Africa. Some linear belts of ultramafic, possibly ophiolitic rocks, trending NE–SW, also traverse the area. No rocks older than 1,100 Ma have been identi-

fied in this shield segment which, at outcrop, is detached from the remainder of the African Precambrian by Phanerozoic cover rocks.

Two possible interpretations of shield development arose during a recent conference at the Institute of Applied Geology at Jeddah* and stimulated considerable debate. The first requires a foundation of older crust, greatly tectonised and thermally overprinted, upon which volcanoclastic sequences were stabilised and through which granites were intruded.

This is the ensialic evolutionary model which has been applied traditionally to mobile belt development elsewhere in Africa. The alternative, ensimatic model, first proposed by Greenwood and others at a Royal Society discussion meeting in 1976, requires the operation of plate tectonic processes with ocean openings and closings at sutures. Protagonists of this model see each suture as the site of a former subduction zone, each tagged with ophiolite and calc-alkaline magma occurrences.

In developing the latter, ensimatic model, correlations in time and space with modern subduction zones led to models being proposed based on South West Pacific back-arc marginal basins and island arcs which would have been marginal to the North East African foreland. Support came particularly from the interpretation of strontium isotopes for granites and associated volcanics (R. J. Fleck, US Geological Survey) which are of mantle-derivation and rather similar to those associated with modern destructive margins. Trends followed by calc-alkaline magmas and their probable subduction-related genesis (W. S. Fyfe, Western Ontario), the interpretation of basic-ultrabasic masses as ophiolitic sutures (A. Al-Shanti, Institute of Geology, Jeddah; I. G. Gass, Open University; R. M. Shackleton, University of Leeds) and the evolution of sediments from deep water marine to shallow marine and continental varieties over 500 Ma (D. G. Hadley, US Geological Survey) were taken to favour ensimatic 'cratonisation' of the Arabian–Nubian shield.

The contrary view, in the broader context of contemporaneous 'Pan-African' evolution elsewhere (A. Kröner, Mainz) was supported by the notion of a geosynclinal cycle, based upon thinned, older continental material under tension. Implicit in this argument is the existence of as yet unrecognised pre-1,100 Ma crust in the Arabian shield, although J. V. Hepworth (Institute of Geological Sciences), S. M. El-Rabaa (University of Khartoum) and J. Vail (Portsmouth Polytechnic) correlated northwards into

Arabia, Mozambiquian structures of the Ugandan and Sudanese areas.

If such ancient cratonic material, thermally and tectonically overprinted with Mozambiquian structures is recognised ultimately, then calc-alkaline magma generation by subcrustal convection with tensional thinning or rifting (as often applied to Archaean crustal features) would be possible. But the existence of older craton does not rule out compressional ensialic plate margin development of Andean type: ensialic development might still be consistent with plate models.

Although considerable data from the Arabian Shield were claimed to support the ensimatic model, an overriding need was recognised for more detailed spatial, geochemical and isotopic studies of all rock types and, particularly, the ostensible ophiolite occurrences. However, the successful application of either island arc or Andean plate marginal models to the late Proterozoic should not surprise us, especially if crustal development is a function of the Earth's exponentially decaying heat productivity: after a week in Jeddah, it seems that Pan-African tectonic styles may well prove to be more closely related to the present day than those of the Archaean. □

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A hundred years ago

WITH reference to a note in *NATURE* (vol. xvii. p. 38) respecting the unflammability of eucalyptus, Mr. A. Nicols writes that this must be a mistake, as in Australia the wood is extensively used as fuel. Acclimatisation of these really valuable trees, Mr. Nicols thinks, should be strongly supported. They yield timber of immense size and strength, durable alike in dry or wet situations, and more proof against the attacks of *termites* than many other woods, and some work up into beautiful furniture. They would probably thrive wherever the mean annual temperature is not below 60°, or, roughly speaking, over an area of about one-half of the habitable region of the earth. From *Nature* 17, 14 March, 391, 392; 1878.

Erratum

The reference to Readhead *et al.* in the article 'Nuclear jet in a radio galaxy' in last week's *News and Views* was inadvertently omitted. It was to the article on page 131 in the same issue (272, 131; 1978).

*February 5–16.

articles

Quiescent cosmology

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The entropy level of the Universe makes it likely that its initial state was isotropic and quiescent rather than chaotic. Only if the equation of state for high density matter tends to the stiff, $p = \rho$, form might such an initially isotropic and homogeneous universe be stable and probable. Of those asymptotic states suggested by strong interaction physics, only for such stiff matter is isotropy compatible with large amplitude gravitational waves, random velocities, vorticity and magnetic fields. An explanation for the microwave background is also possible in such a model.

COSMOLOGISTS since Misner¹ have put much effort into analysing the possible and probable dynamical structure of the early Universe at redshifts $z \gtrsim 10^3$. It was hoped that these investigations might reveal the present structure of the Universe to be logically inevitable. This philosophy, where arbitrarily irregular sets of initial conditions are smoothed into the state of comparative isotropy and quiescence observed today, is known as 'chaotic cosmology'¹⁻³. It has been pointed out⁴, however, that the chaotic cosmology concept is probably untenable in its full generality, both at a classical and quantum level. The present high isotropy and finite entropy of the Universe coupled with the second law of thermodynamics allow only a finite amount of 'irregularity energy' to be dissipated in the past. Hence only a finite degree of irregularity in the initial state is compatible with the $\sim 10^8$ photons per baryon in the microwave background⁵. Specifically, as the shear energy diverges faster than the photon energy on approach to the singularity, $t \rightarrow 0$, where a profusion of efficient dissipative mechanisms are available, the dynamics cannot be dominated by anisotropy there without the overproduction of entropy ensuing.

The logic of this analysis simplifies cosmology by indicating that the Universe must have been of slightly perturbed isotropic FRW (Friedman-Robertson-Walker) character right back to the singularity. But, it also only emphasises the theoretical problem of why such an initially regular state existed, given its low kinematic probability, *a priori*. Further, it seems to generate a paradox: if any large-scale shearing motion, vorticity, peculiar velocity or magnetic field component were present in the Universe today—observed, say, by a characteristic distortion in the spectrum or temperature isotropy of the 3 K background—then the standard radiation-dominated early Universe must have been dominated by the anisotropy energy associated with such irregularities before some redshift, z_s , in the past. As the presence of inhomogeneity in the Universe makes it impossible that large-scale anisotropy does not exist at some level, however small, why then did the dissipation of this anisotropy at times $z > z_s \sim 10^{10}$ not produce excessively more entropy than we now observe?

This paper points out that there is a natural answer to this question which might also explain why the initial state was virtually isotropic and homogeneous. It is claimed that although an isotropic singularity is geometrically and kinematically improbable, it could be thermodynamically preferred if the equation

of state of strongly interacting matter were to take on the 'stiff', $p = \rho$, form at high density. Zeldovich⁶ suggested that this occurs for baryons interacting by a vector meson field. Only for this equation of state is an isotropic singularity completely stable. This approach may also be compared with that of Hawking⁷ regarding the 'energetic' probability of isotropy in a 'space time'. We note that here only the behaviour near $t \approx 0$ is considered. An analysis of the $t \rightarrow \infty$ state of some homogeneous distortions of the non-closed FRW models was made in ref. 59. It indicates that our considerations would explain the late time structure also if the universe were close to a zero binding energy state.

Equation of state at high energy

In equilibrium the general effect of particle statistical thermodynamics is expressed by an equation of state, $p(\rho)$ where $p \equiv$ pressure and $\rho \equiv$ density. At temperatures below $\sim 10^{11}$ K there is a fairly clear understanding but at higher energies this is blurred by the intervention of strongly interacting fields of which our knowledge is very uncertain. Unfortunately, this hadron dominated regime is very important to the cosmologist as the interaction of strong and gravitational forces will mould (or even remove) the structure of the cosmological singularity. Specifically, we expect these considerations to be of interest at times earlier than the time at which the universal density reaches that of nuclear matter, $\rho_N \gtrsim 10^{15} \text{ g cm}^{-3}$; this occurs for cosmic times $t_N \lesssim 10^{-4} \text{ s}$ ($\leftrightarrow Z_N \gtrsim 10^{12}$). From the very detailed descriptions of the various competing models of this era^{8,9}, we may isolate three types of predicted coarse grained structure of matter as $\rho \rightarrow \infty$ and strong interactions dominate $p \rightarrow 0$; $p \rightarrow \frac{1}{3}\rho$; and $p \rightarrow \rho$. We shall comment on each of these asymptotes which are special cases of a general perfect fluid state, $p = (\gamma - 1)\rho$, with sound speed, $c_s \equiv (\gamma - 1)^{1/2}$.

(1) Soft equation of state: $p \approx 0$, $c_s \approx 0$: this asymptotic state could arise in a cold, low entropy early Universe filled with non-interacting baryons^{2,3,10} by extrapolating the standard picture of the cosmic baryon fluid. It is of greater interest that this state results from the Hagedorn composite particle picture¹¹. If this theory of the thermodynamics of strong interactions is correct then something close to the singularity matter would have existed in the form of strange, nonrelativistic massive particles. Hagedorn's model also predicts the existence of a maximum temperature. Since the measured density of mass states seems to have a form $\rho(E) \propto \exp(\beta E)$ with $\beta^{-1} \sim 160 \text{ MeV}$ then in conditions of thermodynamic equilibrium, the average energy of a mass state is given by

$$E_{av} = \frac{\int E \rho(E) \exp(-E/kT) dE}{\int \rho(E) \exp(-E/kT) dE} \quad (1)$$

The exponential form of $\rho(E)$ will prevent the convergence of these integrals if T exceeds $T_{max} \sim \beta^{-1} \sim 160 \text{ MeV}$. Thus, in this picture, energy increase merely spawns new particles rather than increasing the energy of those already present.

(2) Hard, thermal state: $p = \frac{1}{3}\rho \sim n^{4/3}$; $c_s = 1/\sqrt{3}$: this corresponds to the standard radiation dominated 'big bang' of Gamow^{12,13}, extrapolated into the hadron era. It has also been shown¹⁴ to arise if at high density strong interactions become

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negligible allowing a state of 'asymptotic freedom'. In this 'quark-soup' state the long-range interactions are screened out by many-body effects, the weak short-range components are amenable to perturbation theory and the fluid behaves as a collection of black-body radiations.

Also the pressure and energy density of a classical non-interacting gas of number density n and particle mass m satisfy

$$p = \frac{1}{3} nm \langle v^2 (1 - v^2)^{-1/2} \rangle \quad (2)$$

$$\rho = nm \langle (1 - v^2)^{-1/2} \rangle \quad (3)$$

where $\langle \rangle$ shows the appropriate average over the particle distribution¹³. These relations show that for a non-interacting gas $p \leq \frac{1}{3}\rho$.

(3) Stiff, 'Zeldovich' state: $p = \rho \sim n^2$; $c_s = 1$: at super-nuclear densities we expect the nucleon gas to interact strongly; meson exchange is anticipated^{6,15}. This will dominate the high energy behaviour if a transition from nuclear to asymptotically free quark matter fails to occur. To model this behaviour Zeldovich first considered a population of stationary baryons whose interaction is mediated by a spin-1 field. The assumption that the system is stationary generates the Yukawa potential for the potential energy of the interaction, $\phi = gr^{-1} \exp(-r/\lambda)$, where g^2 is the strong coupling constant, and λ the range. The total energy per baryon of mass m is thus,

$$E = m + \frac{1}{2} g^2 n \int \phi d^3x = m + 2\pi g^2 n \lambda^2$$

$$\leftrightarrow p = nm + 2\pi g^2 n^2 \lambda^2$$

and if the range is density independent, the pressure is determined as

$$p = \frac{-d}{dV} (\rho V) = 2\pi^2 g^2 n^2 \lambda^2.$$

Hence, as the density increases, $n \rightarrow \infty$, the potential energy of the interaction dominates and $p \sim \rho \sim n^2$. This type of calculation can only be taken as indicative, as it assumes that the static interaction potential of N baryons can be represented as a collection of two-body potentials. Such an approximation is valid at low densities, but as $\rho \rightarrow \infty$ it will break down and the many-body potentials will dominate the interaction.

The simplified classical model has been improved by Walecka¹⁵⁻¹⁷ who proposed a relativistic, many-body quantum field theoretic model for high density matter incorporating baryons interacting through neutral scalar and vector meson fields. These are taken to represent the long-range attractive and short-range repulsive features of the nucleon-nucleon interaction which are predominantly responsible for nuclear saturation. He also found $p \sim \rho$ at high density.

In a review of experimental and theoretical results pertaining to the behaviour of high density matter in the universe, Canuto¹⁸ claims a tentative preference for the $p = \rho$ asymptote on the basis of high energy p - p scattering experiments¹⁹.

The isotropy of the Universe at high density

We now consider some unique cosmological features and consequences of the Zeldovich state in the arena of the early Universe, $t \lesssim 10^{-4}$ s. By using the early Universe as a 'theoretical' high energy laboratory we shall aim to show how only in the $p = \rho$ thermodynamic state is an initially isotropic and homogeneous universe natural and stable.

Gravitational waves—shear anisotropy

When long-wavelength ($\lambda \gtrsim ct$), gravitational waves are present throughout an evolving matter distribution this wave field determines preferred directions and orientations in space, endowing the space-time with shear anisotropy. If this behaviour is simultaneously realised everywhere then spatial homogeneity may be maintained and such cosmological models with infinite wave-

length gravitational waves contain just homogeneous shear, σ , and expansion $\theta = [\ln(R^3)]^\bullet$ as kinematic features²⁰. These anisotropically expanding configurations are *a priori* far more likely models for behaviour near the singularity than the traditionally employed FRW models because the shear energy, $\sigma^2 \sim R^{-6}$, diverges faster than any perfect fluid energy with sub-photon sound speed, $p = (\gamma - 1)\rho \rightarrow \rho \sim R^{-3\gamma}$, where R is the scale factor²¹. This property allows us to neglect the influence of dust and radiation upon the metric tensor as $t \rightarrow 0$ and study their hydrodynamic non-linearity as a test fluid unencumbered by the problems of gravitational non-linearity. These studies reveal the well-known Kasner and Mixmaster-like vacuum behaviour near the singularity²²⁻²⁴. However, if $\gamma = 2$ and the matter field stiff, then the cosmology may never become dominated by the vacuum anisotropy energy terms as $t \rightarrow 0$ and the possibility of a matter-dominated singularity of quite different character to that envisaged by Belinskii, Lifschitz and Khatatnikov²⁵ is admitted. Specifically, the special interest in this possibility is that if any large scale anisotropy is present in the Universe, however small, then it will have dominated the expansion dynamics before some early time unless $p = \rho$ in the neighbourhood of the singularity. This may be the only way in which shear in the present Universe can be reconciled with the dissipative efficiency of the early Universe⁴ and the present entropy. Alternatively, it is possible to allow the singularity structure to contain the quite general dynamical features of shearing motions on all scales yet maintain a quasi-isotropic state²⁴. Thus, the 'general' solution to the cosmological Einstein equations admits the quasi-isotropic solution as $t \rightarrow 0$ for $p = \rho$ matter but not for other equations of state. If $\sigma/\theta < 1$ when the equation of state stiffens at t_s then $\sigma/\theta < 1$ also for all $t < t_s$. Some detailed examples are;

(1) Flat universe: in vacuum the well known Kasner solution of Bianchi type I is

$$ds^2 = dt^2 - \sum_{i=1}^3 t^{2p_i} dx_i^2; \sum_{i=1}^3 p_i = \sum_{i=1}^3 p_i = 1 \quad (4)$$

thus $-\frac{1}{3} \leq p_1 \leq 0 \leq p_2 \leq \frac{2}{3} \leq p_3 \leq 1$. This simple solution provides a basic building block for more sophisticated irregular cosmological models and is characteristic of a large class of solutions to the field equations. The 'Mixmaster' is composed of dynamics similar to a time matched series of Kasner solutions in which the implosion in the p_1 direction generates sufficient spatial curvature anisotropy to periodically permute its direction of collapse around the three axes in turn. As the implosion phenomenon is generated by the vacuum anisotropy energy, $\sigma^2 \sim R^{-6} \sim t^{-2}$, (up to logarithmic terms), it persists when any perfect fluid with $p < \rho$ is introduced into the dynamics. However, in the stiff case equation (4) changes and the exact solution becomes, ($8\pi G \equiv 1$) (refs 26-29),

$$ds^2 = dt^2 - \sum_{i=1}^3 t^{2p_i} dx_i^2; \sum_{i=1}^3 p_i = 1 = \Sigma p_i^2 + k^2 \quad (5)$$

$$p = \rho = \frac{k^2}{2t^2}; k^2 \in [0, 2/3] \quad (6)$$

In this case the Kasner indices are allowed more freedom because of the matter stress and,

$$-\frac{1}{3} \leq p_1 \leq \frac{1}{3}; 0 \leq p_2 \leq \frac{2}{3}; \frac{1}{3} \leq p_3 \leq 1 \quad (7)$$

Explosive, quasi-isotropic expansion occurs in all directions when there is sufficient matter to insure $k^2 > 1/2$, (the FRW model has $k^2 = 2/3, p_i = 1/3$). In this case matter dominates the singularity. We shall see later that the presence of a magnetic field may even ensure $k^2 > 1/2$ (ref. 28).

Other investigations^{23,30} reveal that a general property of the vacuum Einstein equations may be the Mixmaster-like evolution near the singularity. No investigations were made of the stiff matter case, but from the above solution it is easy to see why the stiff Mixmaster does not oscillate in this fashion. Even if at some

time the rate parameter p_1 were negative, sooner or later the stochastic permutation of the p_i would generate a configuration in which all $p_i > 0$ and thereafter no oscillations could occur. This is the asymptotically stable state.

(2) Closed universe: it is usually possible to adapt known vacuum solutions to generate stiff matter solutions. Such a procedure generalises the Taub universe^{31,32}

$$ds^2 = dt^2 - a^2(t)(d\psi + \cos\theta d\phi)^2 - b^2(t)(d\theta + \sin^2\theta d\phi)^2 \quad (8)$$

$$a^2 = A \operatorname{sech}(A\tau); b^2 = \frac{B^2}{4A} \operatorname{ch}(A\tau) \operatorname{sech}^2\left(\frac{B}{2}(\tau + \tau_0)\right) \quad (9)$$

$$\rho = \frac{M^2}{4a^2b^4} \text{ where } A^2 + M^2 = B^2 \quad (10)$$

$$\text{where } A, M, B, \tau_0 \text{ are constants and } dt = ab^2 d\tau. \quad (11)$$

Using this solution we may follow the Kasner index interchange at a 'Mixmaster' bounce by taking $\tau = \infty$ as the initial epoch and $\tau = -\infty$ the final. The interchange of Kasner indices may be quantitatively evaluated and oscillations are eventually suppressed in the $p = \rho$ solution as expected (see also ref. 33). As $t \rightarrow 0$ this solution asymptotes to an axisymmetric form of universe (1) above.

These investigations might be of interest in the study of the gravitational collapse of finite objects small enough to ensure the absence of horizons. An investigation of the purely general relativistic effects of Mixmaster-like dynamics has not yet been made in such small scale collapses. In particular, the gravitational radiation flux from the object would depend on the equation of state under which this collapse took place³⁴. In view of the above examples, if $p = \rho$ were ever to prevail a far less rapidly varying quadrupole moment would be experienced.

(3) Open universe: here we give a new Bianchi VII_h solution containing stiff matter and circularly polarised gravitational waves generalising the vacuum model of Lukash³⁵

$$ds^2 = dt^2 - g_{\alpha\beta} dx^\alpha dx^\beta; g_{33} = c^2(t) \quad (12)$$

$$g_{\alpha\beta} = a^2 \exp(2z) \begin{pmatrix} \operatorname{ch}(\mu) + \operatorname{sh}(\mu) \cos(kz + \phi), & \operatorname{sh}(\mu) \sin(kz + \phi) \\ \operatorname{sh}(\mu) \sin(kz + \phi), & \operatorname{ch}(\mu) - \operatorname{sh}(\mu) \cos(kz + \phi) \end{pmatrix} \quad (13)$$

$$a, b, = (1, 2)$$

where $a(t)$, $b(t)$, $\mu(t)$, $\phi(t)$ are unknown functions and k is the propagation vector determining the wavelength, λ .

$$\lambda = 2\pi k^{-1} c(t) \text{ and } \omega = 2\pi \lambda^{-1} \quad (14)$$

The specialisation $\mu = 0$ reduces the metric to that of Bianchi V whereas the additional imposition $a = c$ allows only the open FRW model. Looking for outgoing wave solutions of the form $\phi = \pm \int \omega dt$ and solving the field equations we obtain²⁹

$$\phi = -a\xi + \phi_0 \quad (15)$$

$$a^2 = \operatorname{sh}(2\xi); c^2 = D \exp(11\xi/4) \operatorname{cosech}^{3/8}(2\xi) \quad (16)$$

$$\mu = 1/2 \ln(\coth \xi);$$

$$p = \rho = \left(A + B \exp(-4\xi) \right) c^{-2} \operatorname{cosech}^2(2\xi)$$

$$\text{where } \xi = \int_0^t \frac{dt}{c(t)} \text{ and } \quad (17)$$

$$(\alpha - k)^2 - 16B = (\alpha + k)^2 + 16A - 44 = 0$$

the constant D is removable and α , A , B are constrained by the two algebraic relations (17). Again as the singularity is approached the flat solution (1) is obtained with the curvature playing negligible dynamic role.

Velocity and vorticity fields

The anomalous behaviour of cosmic velocity and vorticity fields required to conserve angular momentum in a stiff fluid has already been pointed out³⁶ as a possible way of generating considerable vorticity on large scales from small fluctuations in the early stages of an expanding universe. Here we consider the converse of this result that allows an isotropic background to be unperturbed by the kinetic energy of random motions as $t \rightarrow 0$. Below we give the behaviour of non-relativistic peculiar velocity fields in both isotropic and anisotropically expanding background geometries. The case of relativistic motion in an anisotropic background is not of direct interest here and will be given elsewhere³⁷. The recent analysis of the general type IX model⁶⁰ seems to indicate that the pathologies associated with velocity fields found by Collins⁶¹ and Shikin⁶² are probably artefacts of non-generic homogeneous universes.

By analysis of the conservation equations for particle current and momentum in expanding Kasner and FRW universes it can be shown that velocities and vorticities evolve as follows:

Isotropic background:

$$v \propto t^{-2(4/3 - \gamma)/\gamma}; \rho \propto t^{-2} \text{ for } p = (\gamma - 1)\rho \quad (18)$$

Anisotropic background: the velocities become direction dependent, but as $t \rightarrow 0$ the dynamics are dominated by motions in the 3-direction.

$$v^\alpha \propto t^{\gamma - 1 - p_\alpha}; \rho \propto t^{-\gamma} \text{ for } p = (\gamma - 1)\rho \quad (19)$$

where p_α are the Kasner parameters given by equations (4) and (7) for $p < \rho$ and $p = \rho$ matter respectively. Thus the anisotropic case reduces to the isotropic solution for $\gamma = 2$ and $p_\alpha = 1/3$. Table 1 shows the three physically significant quantities associated with these velocity fields for $p = 0$, $\rho/3$, ρ fluids. These are the 3-velocity, v^α , the angular velocity, ω^α , and the spin contrast parameter $\omega_\alpha^2 \rho^{-1}$ which describes the centrifugal energy relative to the background potential energy.

These results indicate that the 'stiff' singularity is the only one in which isotropy is stable to the presence of velocity fields near $t \sim 0$. Only for this state can small relic v and ω fields exist in the Universe today which will not necessarily dominate the expansion dynamics before some time in the past. Alternatively, only for the $p = \rho$ asymptote is the dynamic generality of velocity and vorticity fields in the initial state compatible with isotropy.

Density inhomogeneities

Many analyses of the behaviour of small perturbations to the isotropic FRW models have been given, (see ref. 38 and references therein) and also of their relation to exact inhomogeneous models³⁹. In general, modulo gauge problems reveal the presence of two types of perturbation mode which grow and decay in time. Usually, discussion is confined to the relatively increasing per-

Table 1 Evolution of non-relativistic velocities, angular velocity and spin energy contrast in isotropic and anisotropic background universes for probable high density states as $t \rightarrow 0$.

Equation of state	Background dynamics	v^α Linear velocity	ω^α Angular velocity	$(\omega^\alpha)^2 \rho^{-1}$ Spin contrast
Stiff $p = \rho$	Iso.	$t^{1/3*}$	$t^{2/3*}$	$t^{8/3*}$
	Aniso.	$t^{1-p_\alpha*}$	$t^{1-2p_\alpha*}$	$t^{4(1-p_\alpha)*}$
Dust $p = \frac{1}{3}\rho$	Iso.	$t^{-1/2}$	t^{0*}	t^*
	Aniso.	$t^{(3/4)-p_\alpha}$	$t^{(3/4)-2p_\alpha}$	$t^{2(1-2p_\alpha)}$
Dust $p = 0$	Iso.	$t^{-4/3}$	$t^{-2/3}$	$t^{-2/3}$
	Aniso.	t^{-p_α}	t^{-2p_α}	t^{1-4p_α}

*Quantities that do not diverge as $t \rightarrow 0$. In this limit the Kasner index, p_α , will tend to p_3 whose variation is given in the text, equations (4) and (7).

turbations as one evolves away from the singularity because of their probable relevance to the origin of galaxies. But, as one moves backwards in time towards the singularity, the relatively decreasing perturbations become relatively increasing. They would thus diverge, falling within their own Schwarzschild radii to produce nonsimultaneous isolated singularities in the Universe. Therefore, should any decaying modes exist in the Universe today, they would have arisen from a chaotically inhomogeneous and anisotropic singularity structure in the past. These presently decaying modes correspond to both primordial shear and 'bang' time fluctuations and indicate that an isotropic singularity is unstable to the presence of such fluctuations. It has also been pointed out by Liang⁴⁰ and Silk⁴¹ that the relatively increasing modes are to be identified with primordial curvature fluctuations. However, in the $p = \rho$ case the perturbation amplitudes have a slightly different property—the relatively decreasing mode becomes constant⁴⁰. Specifically,

$$\frac{\delta\rho}{\rho_0} = A(x) t^{(\gamma-2)/\gamma} + B(x) t^{2(3\gamma-2)/3\gamma} \text{ for } p = (\gamma-1)\rho \quad (20)$$

where t is co-moving proper time, and the fluctuation scale large, $\lambda \gtrsim ct$. It is interesting that a quiescent isotropic singularity of the type usually envisaged is really only completely stable to A -mode fluctuations when $p = \rho$. The physical reason for the constancy of the A -mode in the stiff case is a consequence of its origin in primordial shear fluctuations carrying an energy density $\delta\rho \sim \sigma^2 \sim t^{-2}$ and also $\rho \sim t^{-2}$. Only in the stiff case does the isotropic matter density diverge rapidly enough to compensate the shear energy generated by tidal stresses. This distinctive feature is thus preserved under anisotropic expansion for stiff matter where $\delta\rho/\rho_0 \sim At^{4p_3} + Bt^{2(1-p_3)}$ with $p_3 \in [1/3, 1]$. These small perturbation results may also be recovered as the limit of an exact non-linear model⁴².

Also, with this type of irregularity:

(1) The linear growth rate of the B -mode is greatest for $p = \rho$ fluid thus easing the problem of growing galaxies from 'reasonably small' fluctuations at 'reasonably early times'¹³.

(2) As Lin *et al.*⁴³ show, the formation of primordial black holes is prohibited whilst the Universe is stiff, even if $\delta\rho/\rho \sim 1$ on scales $\lambda \sim ct$. This property is a consequence of the light-like sound speed in the stiff fluid which makes the Jean's length equal the horizon size and the behaviour persists in anisotropically expanding backgrounds^{3,44}.

(3) The maximal sound speed also prevents the formation of shocks having discontinuous variation of fluid variables⁵⁸; thus in conjunction with (2) it gives such cosmologies a natural degree of quiescence, limiting entropy production to linear processes⁴⁵. This property also makes exact analyses of the Einstein field equations simpler than in the presence of $0 \leq p < \rho$ fluids and enables one to study the properties of gravitational non-linearity unencumbered by the simultaneous presence of hydrodynamic non-linearity.

Magnetic fields

We now consider some implications of a primordial cosmic magnetic field. The later observational and theoretical implications of such a large-scale force field are discussed elsewhere⁴⁶. The existence of such a field has often been postulated to explain the presence of galactic fields and it has recently been suggested that it might even determine the morphology of galaxies⁴⁷; the angle between the magnetic and vorticity vectors establishing the Hubble sequence of galaxies. One of the principal arguments against the existence of a sizeable cosmic magnetic field has been that the directionality it imposes upon the space-time would lead to severely anisotropic dynamics at early times in a radiation dominated universe^{27,48-50}. However, a large primordial magnetic field can exist in a completely isotropic space-time if the equation of state is stiff. Doroshkevich²⁸ and Thorne²⁷ give an axially symmetric asymptotic solution for an anisotropic universe

containing a magnetic field, $\mathbf{B}$;

$$ds^2 = dt^2 - A^2(t)(dx^2 + dy^2) - W^2(t)dz^2 \quad (21)$$

$$A = t^p; W = t^{1-2p}; \mathbf{B} = bt^{-2p} \quad (22)$$

$$p = \rho = p(2-3p)t^{-2}; 0 \leq b < \infty; 0 < p < 1/2 \quad (23)$$

and for an isotropic background, the asymptotic behaviour is

$$A = W = t^{2/3\gamma}; p = (\gamma-1)\rho = 4(\gamma-1)/3\gamma^2 t^2 \quad (24)$$

$$\mathbf{B} \equiv 0 \text{ if } \gamma \leq 4/3 \quad (25)$$

$$\mathbf{B} = bt^{-4/3\gamma} \text{ if } 4/3 < \gamma \leq 2 \quad (26)$$

There are two important features of these solutions. First, that for stiff fluids an isotropic background geometry may accommodate a large-scale magnetic field with test-matter type behaviour $B^2 \sim t^{-4/3}$. By contrast, dust and radiationlike initial states require anisotropic dynamics to accommodate the field stresses. In addition we see from equations (21–23) the very surprising result is obtained that if a magnetic field is present all the Kasner indices are positive as $p < 1/2 \leftrightarrow k^2 > 1/2$ in equation (6), (this behaviour need not necessarily occur in the non-axially symmetric case, however)²⁶. At first this might seem unusual because of the dynamically negligible role of a radiation fluid in this background, but its anisotropic pressure can lead to significant stresses in some directions which modify the dynamics to quasi-isotropic form. If we examine the energy contrast parameter, $B^2\rho^{-1}$, we find in isotropic backgrounds $B^2\rho^{-1} \sim t^{-2/3\gamma(4-3\gamma)}$. Thus, when $\gamma > 4/3$ the magnetic stresses do not dominate, $B^2\rho^{-1} \rightarrow 0$, as $t \rightarrow 0$, and the familiar analogy with vorticity is also borne out, (compare Table 1).

Finally, we consider how the vortical instability present in $p = \rho$ fluids, $v \propto \omega^2 \propto t^{2/3}$, might allow the spontaneous generation of a large turbulent magnetic field in the manner first envisaged by Batchelor⁵¹. The diffusive properties of the vortical flow ensure that neighbouring fluid elements gradually move apart. Consequently, if a 'seed' magnetic field is present initially and the fluid is highly conducting, the frozen magnetic lines of force will tend to be extended by the diffusive motion of the fluid and increase the magnetic field strength. This amplification process is expected to be present in any magnetohydrodynamical flow in competition with the magnetic dissipation by Joule heating. The imbalance of these effects determines whether or not the average field grows or decays in time. Batchelor's Newtonian analysis could be extended to relativistic flows using the equations of Ellis²¹. Analogously, we must determine the sign of the Batchelor number, $\beta \sim \Sigma v c^{-2}$ in the fluid, where Σ is the conductivity, v the kinematic viscosity and c the light speed. If $\beta \gg 1$ the dissipative losses will be unable to compete with the field amplification. The essential point about this behaviour concerns the energy spectrum of the resulting magnetic field but unfortunately there is a marked difference of opinion on this. It was originally thought that ultimately equilibrium is reached with the small wavelength components (which contain the bulk of the vorticity) containing comparable amounts of kinetic and magnetic energy; magnetic stresses then stiffen the fluid and prevent further growth. But Biermann and Schlüter⁵², Kraichman and Nagarajan⁵³ argue that the pile up of magnetic energy on small scales alters the vorticity spectrum there and equipartition is gradually achieved on increasingly larger scales; ultimately the magnetic energy equals the kinetic energy of the largest eddies. In the cosmological problem it is this latter possibility that is of interest for field generation in the early Universe if there is sufficient time for it to occur. In the vicinity of the cosmological singularity microscopic seed fields can be expected to arise through statistical fluctuations or battery effects related to spinning particles⁵⁴—the latter would be more efficient in a mixture of stiff, rather than soft, baryons and radiation. This can be amplified if $\beta > 1$ in the stiff fluid. Checking the effective value of

β is possible only on vague dimensional grounds. In natural units, ($8\pi G = c = \hbar = 1$), we have for $\beta \sim v\Sigma$. Suppose the collision time approximation is adequate, then, $v \sim \rho t_{\text{coll}} \sim \rho(\sigma n)^{-1}$ with σ the cross-section for collisions and ρ the baryon density related to the number density by $\rho \sim n_p^2 m_p^{-2}$, where 'p' denotes proton. Taking $\sigma \sim m_p^{-2}$ we obtain $v \sim n_p$. Assume the currents are carried by charges Q (these may be electrons or vector mesons). Then $\Sigma \sim n_0 Q^2 t_0 m_0^{-1}$ where the free time $t_0 \sim (\sigma_p n)^{-1}$ and $\sigma_0 \sim m_0^{-2}$. Thus, $\Sigma \sim m_0 Q^2$ where $Q^2 \sim 10^{-2}$ for electrons and ~ 10 for mesons. Therefore, $\beta \sim \Sigma v \sim n_p Q^2 m_0 \gg 1$ close to the singularity as $n_p \rightarrow \infty$ there, and amplification may occur.

Entropy

The stiff state for baryons near the singularity is also attractive as it permits an explanation of the microwave background's existence. This is equivalent to explaining the entropy per baryon of the Universe⁵⁶, S_b , and was first pointed out by Zeldovich⁵⁵. The dissipation of small amplitude, $\delta\rho/\rho \sim 10^{-4}$, density fluctuations entering the horizon during the period $t_{\text{pl}} \sim 10^{-43}$ s, to $\sim 10^{-33}$ s, after the 'bang' could generate $S_b \equiv s/kn_b = 4aT^3/3kn_b \sim n_\gamma/n_b = (\pi/13)(k/\hbar c) T^3 \sim 10^8\text{--}10^9$ by the end of the stiff era. A fluctuation with this amplitude on every scale entering the horizon might also explain the origin of large-scale structure in the Universe⁵⁵. Liang has also shown that a similar entropy production could arise in an anisotropic model by shear damping. Both mechanisms are unfortunately extremely sensitive to the period of damping and the global dynamics⁴.

Conclusions

The Zeldovich hypothesis of a stiff equation of state at high energy can introduce a large number of distinctive features into the structure of the very early Universe during the hadron era. It is particularly appealing that all these are simplifying features and might provide some clues as to why the Universe displays such characteristically regular structure on large scales today, given the enormous number of kinematically more favourable degrees of freedom open to it. Earlier work has indicated that the initial state is probably isotropic⁴ up to small fluctuations. Should we therefore conclude that nature prefers quiescent to chaotic cosmology? If so, then it is possible that certain thermodynamic states are more cosmologically compelling than others. This paper claims tentatively that the $p = \rho$ behaviour represents such a natural initial state. Our conclusions are, therefore, first, the general behaviour of cosmological solutions to Einstein's equations^{24,25} discovered for vacuum, dust and radiation dominated models does not persist in the presence of stiff fluids, and a quasi-isotropic behaviour is admitted as an open neighbourhood in the superspace of solutions to these equations. Second, the singularity structure is stably quiescent. This means that density, velocity or gravitational distortions do not exist from the FRW isotropic models which diverge as $t \rightarrow 0$. This contrasts sharply with the other equations of state. It suggests that a simultaneous singularity containing just small, quasi-statistical curvature, shear and velocity perturbations can only arise naturally in the $p = \rho$ thermodynamic state. Other states require that these divergent modes be postulated to be absent for no good reason. Third, the growth of vorticity and velocity perturbations in time allows vorticity to exist naturally in the Universe on large scales. Magnetic fields might also be amplified in this way and additionally a large primordial field may exist in an isotropic background when $p = \rho$. This is not possible for $p \leq (1/3)\rho$ states. Fourth, on a physical level the absence of shock waves and primordial black hole formation places a natural limit on the admissible degree of hydrodynamic non-linearity possible near the singularity. On a mathematical level this absence of characteristic crossing, in conjunction with a minimum of characteristic

length scales, makes for easier analysis of complex dynamical configurations by ensuring virtual self-similarity. Finally, there is the possibility of generating the specific entropy of the Universe by dissipation of small amplitude irregularities in the matter energy near the singularity, although special initial conditions might be necessary to curb the high efficiency of this process, if the observed level is not to be overstepped. For universes having radiation-like states near the singularity the specific entropy currently remains an unexplained initial condition, whilst those having soft, $p \approx 0$, form require extremely anisotropic dynamics to explain it, as there is insufficient rest mass energy in the matter field to generate the observed entropy before a redshift, $z \sim 10^4$. Such enduring irregularity would conflict with observations of light elements, (He^4 , D)⁵⁷, and the microwave background structure^{3,13}.

In the light of this circumstantial and indirect evidence it is hoped that further detailed investigations will be made into the status of the Zeldovich equation of state by high energy physicists and that new theoretical 'experiments' may be devised by cosmologists to investigate its implications in the singular environment of the early universe.

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Organic geochemical indicators of palaeoenvironmental conditions of sedimentation

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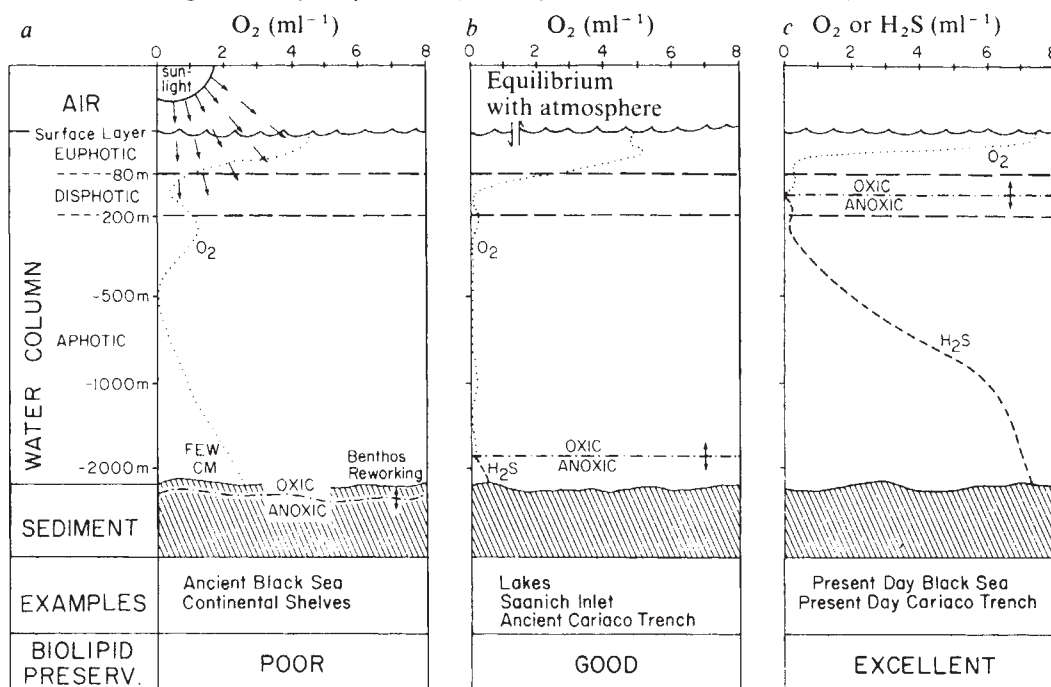
Our increasing knowledge of the geochemical processes of present-day sedimentation conditions provides a basis for evaluating the role of oxic and anoxic sedimentary environments. In particular, there are organic geochemical parameters, such as lipid composition, sulphur and organic pigments, which link Recent sediments with their geologically older counterparts, and these may therefore be used to assign the depositional palaeoenvironments of ancient sediments and petroleum.

THE lipids of Recent and ancient sediments reflect the original sedimentary organic input and the subsequent processes of alteration to which they have been subjected. Initially, diagenetic processes involving microbial degradation¹ and chemical conversion² are conditioned by and may influence the depositional environment. Thus, both redox potential³ and acidity⁴ are key factors in the preservation of organic matter. During subsequent maturation, which accompanies lithification, the temperature⁵, pressure⁵ and the petrology (in particular the clay content⁶) of the sediment are the major controls on its lipid composition.

The available data suggest that the oxicity/anoxicity of the sediment and the water column affects the amount and nature of the organic matter incorporated into the sediment during deposition and hence the composition of geolipids ultimately preserved. The role of organic geochemistry ought, therefore, to include assessment of the oxicity/anoxicity of Recent and ancient depositional environments. In particular, we propose as an extension of the studies by Brooks *et al.*⁷ and Powell and McKirdy⁸ that the pristane to phytane ratios (Pr/Ph) of ancient sediments and oils reflect their palaeoenvironmental conditions of sedimentation.

For some time the relative inputs of marine and non-marine organic matter to sediments, source rocks and petroleum have been assessed from specific compounds (molecular markers) in their geolipids. Crude oils associated with non-marine input have a higher wax (long chain alkane) content than marine crudes⁸⁻¹⁰. Thus, a high wax content is thought to indicate a depositional environment where a marked terrigenous component has been incorporated into the sediment. The basis for this inference is the fact that higher plants—which would be expected to make significant contributions to land-derived organic matter—biosynthesise *n*-alkanes of greater chain length¹¹⁻¹³ (for example,

Fig. 1 Schematic representation of typical sedimentary environments (adapted from Horne³³). The water column is divided into three zones; the euphotic zone (where photosynthesis occurs), the disphotic zone (with only dim light and no effective plant production) and the aphotic zone (absence of light and no plant production, but only carnivores and detritus feeders). Three levels of the oxicity/anoxicity interface (— · — · —) are shown; *a*, in the sediment at a depth of a few cm³⁴, oxic; *b*, in the bottom waters or at the sediment/water interface, oxic/anoxic; *c*, in the disphotic zone, anoxic. The oxygen concentrations (· · · · ·) against depth for (*a*) and (*b*) are oceanic examples from Richards³⁵ after Bruneau *et al.*³⁶ with an oxygen minimum in the disphotic zone and, for (*a*) another at greater depth. *c* Shows the O₂ (· · · · ·) in the oxic zone) and H₂S (— · — · —) in the anoxic zone) for the eastern Black Sea as reported by Fonselius³⁷. The examples of these different sedimentary environments quoted are fully discussed in the text. The values of the biolipid preservation are intended to give only a relative scale of the quality of preservation, although this can also be expected to relate to the quantity.



C₃₁) than marine phytoplankton, the dominant *n*-alkane of algae being C₁₇ (refs 14-16).

Similarly, polycyclic triterpenoids are found in petroleum¹⁷ and their presence was at first taken as evidence of non-marine contribution^{18,19}, as they had been detected in the lipid extract of ferns²⁰⁻²⁵. However, triterpenoids have now been isolated as minor constituents of marine micro-organisms (for example, algae²⁶ and bacteria²⁷⁻³⁰) and therefore can no longer be considered as definite indicators of terrestrial input.

The depositional palaeoenvironments of sediments and source rocks which generate non-marine petroleum have been described as deltaic³¹, where a higher contribution of land-derived organic matter can be expected³¹. Such environments may show lower amounts of *n*-heptadecane (relative to other lipids) than those laid down under fully marine conditions, due to a greater dilution of the algal lipid input by other biotic sources. This argument augments Lijmbach's³² proposal that the Pr/*n*-heptadecane ratio of a crude oil may give an indication of its depositional origin, distinguishing open water conditions of sedimentation (values < 0.5) from inland peat-swamp environments (values > 1.0).

Oxic/anoxic sedimentary environments

Figure 1 is a schematic representation of three sedimentary environments which differ in the position of their oxic/anoxic interfaces as reflected in their dissolved oxygen content against depth.

The major proportion of the autochthonous marine biomass produced in the oxic portion of the water column consists of phytoplankton³³. Phytoplankton contain approximately 3% (dry weight) of chlorophyll, based on their organic carbon content, which amounts to about 1% phytol and 2% chlorins³⁸. The eventual input of these compounds to the underlying sediments depends on the sinking rate and predation. The sinking rates reported for phytoplankton are extremely variable and range from 1 m to 1 km d⁻¹ (ref. 39). Bacterial degradation and predation through the pelagic food web is also variable⁴⁰. However, there is a net downward flux of autochthonous organic debris from the euphotic zone. In an anoxic environment (see Fig. 1c and Fig. 2c) this downward flux includes up to 20% of the organic carbon generated in the euphotic zone, while in oxic depositional conditions (Fig. 1a and b, and Fig. 2a) it represents only about 2% of the organic carbon⁴¹. Superimposed on this autochthonous input to marine sediments is allochthonous organic matter supplied by water-transport, eolian fallout, or ice-rafted material⁴². The contribution of allochthonous biolipids similarly depends on their sinking rate and on predation and transport parameters. In certain areas such as continental slopes, the contribution of allochthonous organic matter is high^{42,43}, while in other areas, (for example, Walvis Bay⁴⁴) it is almost zero. The biolipid input to a sediment will, therefore, depend on its origin and may be indicative of its source.

In oxic sedimentary environments (Fig. 1a) labile biolipids such as sterols⁴⁵, carotenoids⁴⁶ and chlorins⁴⁷ will be destroyed rapidly, or show major changes reflecting the oxic conditions of their deposition. In anoxic sedimentation conditions (Fig. 1b, c) these biolipids will tend to be preserved, generating sediments with higher contents of these compounds and of their closely related degradation products which still conserve characteristic structural features derived from biosynthetic processes⁴⁸. Anoxicity decreases predation and bacterial degradation throughout the water column, preventing extensive oxidative alteration of the organic biomass before sedimentation, so that a greater proportion of organic matter reaches the sediment³². High values of organic carbon (> 10%) for sediments have therefore been equated with anoxic conditions of sedimentation⁴⁹. In contrast, high values of calcium carbonate seem to indicate a sudden lowering of the O₂-H₂S interface⁵⁰. However, Richards⁵¹ has suggested that the enhanced preservation of organic matter observed in anoxic marine environments is a

reflection of faster rates of sedimentation and accumulation rather than slower rates of organic decomposition. Since bacteria are concentrated at the sediment/water interface of both oxic⁵² and anoxic⁵³ sediments, a rapid sedimentation rate will reduce the residence time of organic matter in the surface zone⁵⁴ and, hence, lessen the opportunity for bacterial alteration to occur. Fluctuations in the organic carbon content of sediments have been taken to be representative of the variations in sedimentation rate⁵⁵ and organic productivity^{55,56}, while Strumm and Morgan⁵⁷ have suggested that the water column anoxicity may be the direct result of organic enrichment in the euphotic zone. Such enrichment in highly fertile and productive surface water increases the amount of organic matter settling through the water column. The oxidation of this large volume of organic matter results in a significant reduction of the dissolved oxygen content, so that anoxic conditions may be produced in the disphotic zone⁴⁹ (Fig. 2b), while the lower waters remain oxic. Hence, anoxic sedimentation may occur where the water/sediment interface lies within the oxygen depleted zone. The northwestern Indian Ocean is a present-day example of such an environment, with an anoxic water column between 150 and 800 m depth⁵⁸. However, the anaerobic sediments formed along its continental margin⁵⁹

Fig. 2 Near-shore environments (not to scale), adapted from Fleming and Revelle⁶³ and Thiede and Van Andel⁴⁹. Three different examples of near-shore environments are given: a, oxic waters and sediments; b, anoxic mid-column waters, giving anoxic sediments on rise; c, anoxic basin waters and sediments. The topographic distinction between a and c is the presence of a sill (for example, as at Saanich Inlet⁶⁸) in the latter, which creates a zone of restricted water circulation, giving rise to the anoxic bottom waters. There are contributions to all of the sediments from allochthonous and autochthonous organic matter, with the former input being derived from terrigenous and eolian sources, while the latter is generated by biota in the euphotic zone. In b the organic productivity is particularly high, causing anoxic mid water conditions⁴⁹. In a smaller amount of organic matter (compared with b and c) passes down from the euphotic zone into the aphotic region (as shown by the split arrows), while the remainder is recycled. However, as little as 20% of the organic matter reaching the bottom water may actually become incorporated into the sediment⁴¹.

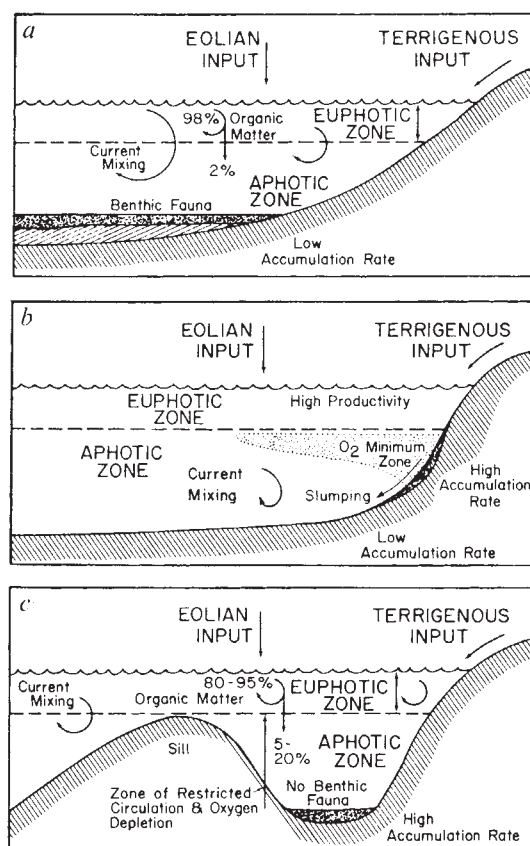


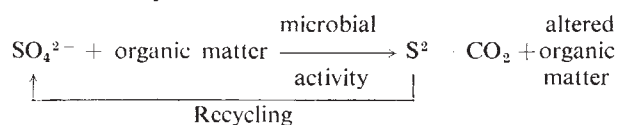
Table 1 Characteristics of Recent sediments and their depositional environments

Sample and depth (m)	Approximate age (yr BP)	Organic carbon (% dry wt)	Total sulphur (% dry wt)	Pr/Ph*	Chlorin† content (µg g ⁻¹)	Depositional environment‡		Refs
						Oxicity/anoxicity	Location	
Cariaco Trench ⁴²-DSDP-15								
147B-1-1 (2)	2,000§	4.3	ND	0.93	ND	Anoxic	Marine	42
147B-1-3 (4)	5,000§	3.7	ND	0.54	460	Anoxic	Marine	42, 47
147B-1-4 (5)	10,000§	3.4	0.4	1.1	ND	Oxic-anoxic	Marine	42, 76
147B-2-1 (11)	20,000§	2.0	ND	ND	1,170	Oxic-anoxic	Marine	47
147B-4-1 (33)	56,000§	2.1	ND	0.74	ND	Oxic-anoxic	Marine	42
147B-5-0 (40)	80,000§	1.3	ND	1.04	1,775	Oxic-anoxic	Marine	42, 47
147B-5-6 (50)	100,000§	1.1	ND	1.5	1,785	Oxic-anoxic	Marine	42, 47
147B-7-4 (67)	130,000§	1.6	0.9	0.61	82	Oxic-anoxic	Marine	42, 47, 76
147C-2-3 (107)	200,000§	1.3	ND	ND	160	Oxic-anoxic	Marine	47
147C-3-1 (138)	270,000§	2.7	1.3	0.86	ND	Oxic-anoxic	Marine	42, 76
Black Sea ⁸³-AII 49								
Random (seabed)	Recent	2.8	1.5	ND	44,000	Anoxic	Marine	42, 77
1461K (0.4)	3,000§	18.0	0.8	0.85	ND	Anoxic	Marine	42, 78
1461P (0.7)	6,500§	NA	1.0	ND	2,200	Anoxic	Marine	42, 77
1462 (1.9)	9,300§	0.6	0.7	0.60	ND	Oxic-anoxic	Lacustrine-marine	42, 78
1462 (2.95)	14,000§	0.3	0.35	1.04	ND	Oxic	Lacustrine	42, 78
1462 (5.0)	16,500§	0.3	0.4	1.09	ND	Oxic	Lacustrine	42, 78
1474 (8.55)	20,000§	0.4	< 0.02	1.43	ND	Oxic	Lacustrine	42, 78
1474 (11.55)	22,830§	0.5	0.06	1.20	ND	Oxic	Lacustrine	42, 78
North Atlantic Ocean ⁸¹-DSDP-12								
114-5-5 (507)	Pliocene	0.15	0.15	0.3	ND	Unknown	Marine	42
Saanich Inlet, British Columbia⁷⁹								
Subsurface (0.15)	Recent	4.8	NA	> 3.0	14	Oxic-anoxic	Fjord	79

NA, not available; ND, not determined.
 * Pr and Ph concentrations determined from gas chromatograms of branched/cyclic hydrocarbon fraction.
 † Expressed as µg per g of organic matter (– 1.3 × organic carbon).
 ‡ Palaeoenvironmental assignments based on the evidence of the fossil record, clay and mineral species.
 § Age inferred from fossil records and sediment accumulation rates^{80,83}, except for the sample at 11.5 m from the Black Sea, for which a ¹⁴C age is given⁸⁴.
 || Expressed as µg per g dry sediment.

do contain indigenous benthic foraminiferal faunas⁶⁰. Depletion of oxygen in the mid water column is also found along the western North and South American continental margin⁶¹. Other factors which influence the extent of decomposition of organic matter include the current energy (linked to the rate of settling) and the degree of bottom water circulation^{56,62}, since restricted circulation can lead to stagnation and oxygen depletion. The overall flux of organic matter through the water/sediment system is, therefore, dependent on the submarine topography (as shown in Fig. 2a and c) as much as on the autochthonous and allochthonous inputs of organic carbon and the rate of deposition. Additionally, the degree of degradation of organic matter effected by sulphate-reducing bacteria, which are widely and abundantly distributed in the marine environment, especially in sediments⁶¹, will depend on the supply of sulphate ions. However, the rate of sulphate reduction is independent of the sulphate ion concentration above certain values⁶⁵⁻⁶⁷ when it becomes a zero-order reaction⁶⁸⁻⁷⁰. A possible limiting factor for this reduction may then be the amount of organic matter available for metabolism⁷¹.

In anoxic environments the sulphide ions produced by the sulphate-reducing bacteria can be oxidised back to sulphate (for example by sulphate-oxidising bacteria such as *Chlorobium* and *Thiobacillus*⁷²), during periods of overturning or in the upper portion of the anoxic zone by vertical mixing across the oxic/anoxic boundary⁷³.



The reduced circulation in anoxic basins (Fig. 2c) means that the sulphide ions are more likely to be incorporated into the sediment in an anoxic environment compared with oxic conditions. Sediments with high total

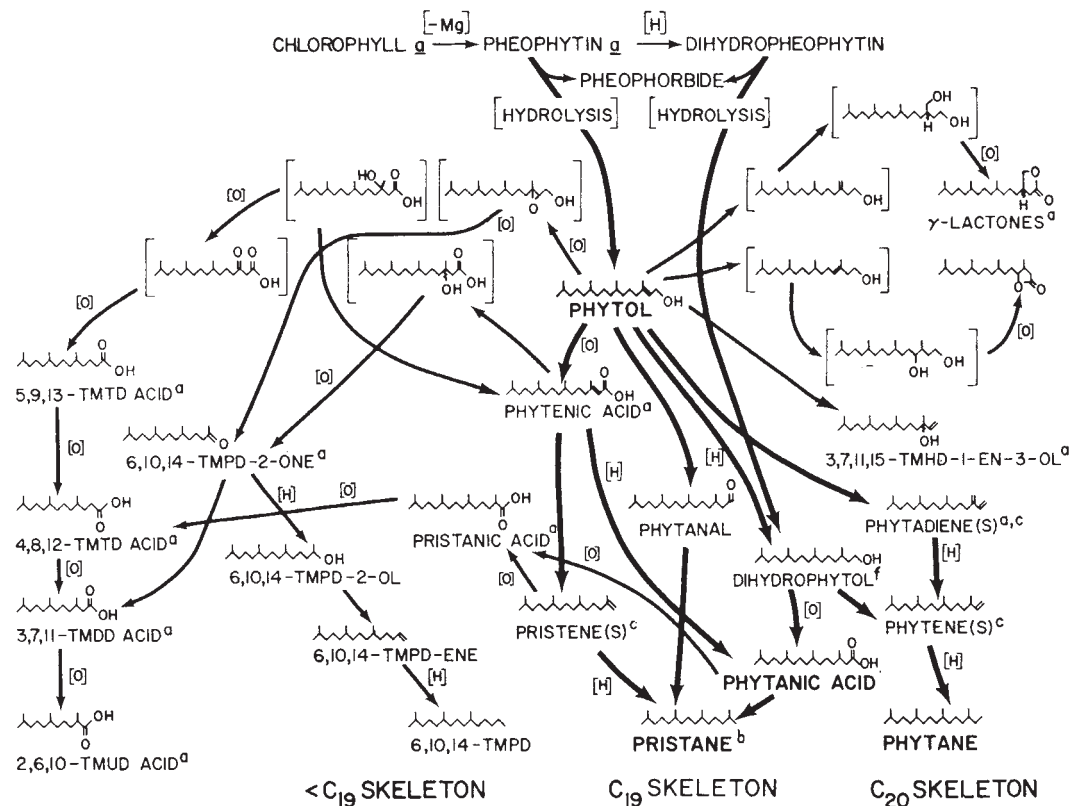
sulphur contents (that is > 1% dry weight) are therefore usually indicative of anoxic sedimentation conditions.

Additional information can be provided by palaeontological and palynological^{73,71} studies of sediments which may enable some estimation of the relative amounts of autochthonous and allochthonous inputs. Also, the total populations⁷⁵, species and diversity^{75,61} and physical characteristics^{75,61} of benthic organisms may be indicative of the oxicity/anoxicity of the sediment while pelagic organisms could similarly indicate the state of the water column.

Recent sediments

Table 1 is a compilation of data for Recent sediments from two anoxic basins (the Cariaco Trench and the Black Sea) and two other sites. The Cariaco Trench is a graben on the Continental Shelf off Venezuela which at present has anoxic bottom waters and is receiving mainly marine sediments⁶⁰. The samples listed were obtained during Leg 15 of the Deep Sea Drilling Project (DSDP)^{42,76,80}. The bottom waters of the Black Sea became anoxic about 7,300 yr ago and the oxic/anoxic interface has been rising ever since⁸¹. The sediments sampled all contain large, allochthonous, terrigenous contributions^{12,75,82} and were obtained during Cruise 49 of the RV Atlantis II, operated by the Woods Hole Oceanographic Institution⁸³. The other samples are: first, a glauconitic, silty clay DSDP sediment⁸¹ from the North Atlantic Ocean, with organic matter of mainly terrigenous origin⁴² (glauconite is typically found in aerobic sediments of shallow to intermediate water depths⁸⁵⁻⁸⁷, but may also be indicative of the presence of reducing microenvironments⁵⁵); and second, sediment from Saanich Inlet, an intermittently stagnant fjord, where the bottom waters become anoxic in late summer^{79,88}, with a high content of organic matter, mainly due to terrigenous input. The incomplete data for these Recent sediments given in Table 1 indicate that those sediments

Fig. 3 Postulated diagenetic pathways for the fate of the phytol side chain of chlorophyll *a* in aquatic environments. These microbial and geochemical transformations have been demonstrated or postulated by Borgohain⁹⁶, Ikan *et al.*⁹⁷, Brooks⁹⁸ and Cox⁹⁹. The isoprenoids are named trivially, where possible, or by the shorthand method of Brooks⁹⁸ (for example, 3,7,11,15-tetramethylhexadec-1-en-3-ol becomes 3,7,11,15-TMHD-1-en-3-ol). Initial reduction (shown by [H]) of phytol leads towards the right hand side of the diagram, preserving the C₂₀ skeleton and hence favouring formation of phytane, whereas initial oxidation (shown by [O]) tends to the centre and left of the diagenetic scheme giving pristane, with a C₁₉ skeleton, and lower isoprenoids. The major pathways to pristane and phytane formation are emphasised by the thicker arrows. *a*, Compounds formed as radiolabelled products from the incubation of U-¹⁴C phytol in sediments (Brooks⁹⁸). The postulated intermediates of these reactions are shown in square brackets. *b*, The formation of pristane from the phytol side chain of chlorophyll *a* is supported by stereochemical studies^{93,95}. *c*, The double bond positions shown are not diagnostic, as several isomers of phytadienes and pristenes (neophytenes) have been found in deep sea sediments^{78,101} while five phytene isomers have been reported in algal mats¹⁰². This figure excludes the direct sediment inputs of pristane, pristenes or phytadienes from zooplankton¹⁰³⁻¹⁰⁵, and the possibility of bacterial phospholipids, rather than chlorophylls, acting as a source for phytol, phytane, phytanic acid and dihydrophytol¹⁰⁶⁻¹⁰⁸, a process which is thought to occur in the Dead Sea¹⁰⁹. Both of these alternative origins for isoprenoids are only indirectly related to the oxicity/anoxicity of sedimentation, hence their exclusion from this simplified diagenetic view.



deposited in permanently anoxic sedimentary basins tend to possess high contents of organic matter (in particular chlorins), and sulphur, together with low pristane to phytane ratios (Pr/Ph). The North Atlantic DSDP sediment is an exception which may be a reflection of microenvironmental conditions⁵⁵, since the existence of reducing microniches within oxidised sediments has been recognised and quantitated^{89,90}. The higher value for Pr/Ph observed in the Saanich Inlet sample presumably arises because the anoxic conditions are only intermittent and the rate of sedimentation is too slow to prevent oxidation when the bottom waters return to oxic conditions.

The correlation of the pristane to phytane ratio with the palaeoenvironment may be assessed from Table 1. Recent sediments deposited in anoxic palaeoenvironments, consisting of an anoxic water column above anoxic sediments (Fig. 1c) have low Pr/Ph values (rather less than unity). Alternating oxic and anoxic conditions of sedimentation or fluctuations in the depth below sea level of the oxic/anoxic interface (Fig. 1b) may be reflected in Pr/Ph values of about 1. Such values may also indicate oxidation during the early stages of chlorophyll degradation in the upper column of oxic water. Wholly oxic conditions (Fig. 1a) such as existed during the deposition of the deeper samples from the Black Sea⁴¹, produce Pr/Ph values rather greater than 1.

Anoxic conditions evidently preserve the C₂₀ isoprenoid skeleton, giving low Pr/Ph values, while oxic conditions cause greater degradation so that the C₂₀ skeleton is less likely to survive intact in the sediment, leading to a high Pr/Ph ratio. Both pristane and phytane are thought to originate from the geochemical diagenesis of phytol^{91,92}, a process supported by the evidence from stereochemical studies of pristane isolated from geological sources⁹³⁻⁹⁵, and laboratory investigations of thermal alteration⁹⁶. Pristane is formed from phytol by an oxidative

pathway, for example via phytanic and/or phytenic acids and subsequent defunctionalisation, while phytane is generated through various reductive paths⁷. These reactions are shown in the schematic representation of phytol diagenesis given in Fig. 3. The Pr/Ph ratios determined for Recent sediments (Table 1) can therefore be explained by the proposal of Welte and Waples¹¹⁰ that in reducing (anoxic) environments reductive processes prevail over decarboxylation, affording low Pr/Ph values, while in oxic conditions the opposite situation exists. Similarly, an anoxic environment will tend to preserve the chlorophyll macrocycle as chlorins, which are converted to porphyrins in ancient sediments by a diagenetic process simulated by the heat treatment of a homogenised sediment¹¹¹. In contrast, in oxic conditions, chlorophyll type pigments are quickly degraded giving rise to sediments with low chlorin contents⁴⁷. Therefore, in Recent sediments, a low pristane to phytane ratio and high chlorin and sulphur contents are indicative of an anoxic environment. Conversely, high pristane/phytane values with low chlorin and sulphur contents indicate oxic environments.

Ancient sediments and oils

The organic content of ancient sediments and petroleum should reflect the original biolipid input, the conditions of deposition and, possibly, the extent of subsequent maturation processes and of any microbial degradation. Table 2 is a compilation of data for various ancient sediments and oils, giving the values of those parameters discussed above with reference to Recent sediments (see Table 1), except that diagenetic and maturation processes have converted the chlorins to porphyrins.

The Green River Formation oil shale and the Messel shale were laid down in anoxic conditions and have suffered little thermal alteration^{123,124}. Their low Pr/Ph values and high

Table 2 Characteristics of ancient sediments and petroleum and their depositional palaeoenvironments

Area, sample (depth/location)	Geological age	Organic carbon (% dry wt)	Total sulphur (% dry wt)	Pr/Ph*	Porphyrin content ($\mu\text{g g}^{-1}$)†	Palaeoenvironmental data‡ Depositional environment	Oxicity/ anoxicity	Refs
Ancient sediments								
Green River Formation Oil Shale	Eocene	17–36	0.30–1.26	0.56	55	Lacustrine	Anoxic	112–115
Messel Shale	Eocene	25–30	0.75–2.8	0.68	50	Lacustrine	Anoxic	116–119
Posidonia Shale	Jurassic	9.52	1.34	2.4	< 5	Marine	Oxic	42
La Luna Shale (Mara Outcrop)	Cretaceous	10.96	0.41	0.45	55,900	Marine	Anoxic	120
La Luna Shale (36–2, 5,000 m)	Cretaceous	12.42	0.17	0.55	2,730	Marine	Anoxic	42, 120
Atlantic Ocean-DSDP-40 364-43-1 (130–133 cm)	Cretaceous (Aptian)	7.8	2.07	0.6	65	Marine	Oxic-anoxic	
Atlantic Ocean-DSDP-41 367-19-4 (10–15 cm)	Cretaceous (Cenomanian)	9.0	1.22	0.3	6	Marine	Oxic-anoxic	
Petroleum								
Boscan Crude 8E4	Eocene§	—	5.13	0.50	3,720	Marine	Anoxic	42, 120
Boscan Crude BN4	Eocene§	—	4.38	0.50	3,540	Marine	Anoxic	42, 120
La Paz Crude P187	Cretaceous	—	3.01	0.66	628	Marine	Anoxic	120
Burgan Crude (Kuwait)	Cretaceous	—	NA	0.70	220	Marine	Anoxic	42, 113
Wilmington Crude (USA)	Miocene	—	1.59	1.2	570	Marine	Anoxic	113, 121
Pembina Crude (Canada)	Cretaceous	—	0.25	2.0	4	Marine	Oxic-anoxic	121 ¶
Redwater Crude (Canada)	Devonian	—	0.47	1.4	40	Marine	Oxic-anoxic	121 ¶
Daniel Crude (Chile)	Cretaceous	—	0.12	1.9	< 2	Deltaic	Oxic	122 ¶
Dicky Crude (Chile)	Cretaceous	—	0.11	2.9	< 2	Deltaic	Oxic	122 ¶

NA, not available.

*Pr and Ph concentrations determined by gas chromatography.

†Expressed as metalloporphyrins in μg per g of extract (for ancient sediments) or of total oil.

‡Palaeoenvironmental assignments based on the evidence of the fossil record, clays and mineral species.

§Age of reservoir rock.

||Unpublished results (B. R. T. Simoneit).

¶Unpublished results (B. M. Didyk).

porphyrin contents, by applying the criteria outlined above for Recent sediments, are also characteristic of anoxic sedimentation. The Posidonia shale, in contrast, has a high Pr/Ph ratio and low porphyrin content, typical of oxic sedimentation. The Boscan and La Paz crude oils are believed to have evolved from the La Luna Formation, a shaly limestone deposited in an anoxic environment¹²⁵. The low Pr/Ph ratio and high porphyrin and sulphur content of these samples are consistent with an anoxic palaeoenvironment of deposition of their source rock. The Burgan and Wilmington crudes are further examples of agreement between the anoxic depositional environment¹²⁰ and organic geochemical parameters; low Pr/Ph and significant porphyrin contents. The intermediate values for Pr/Ph and porphyrin content of the Pembina and Redwater crudes also correlate with their inferred transitional origin. The Daniel and Dicky crudes were deposited in oxic deltaic conditions (B.M.D., unpublished results), which concurs with the assignment on the basis of a high Pr/Ph ratio and low sulphur and porphyrin contents. The two Cretaceous DSDP samples show Pr/Ph ratios less than unity, which agrees with their partially anoxic origin.

Powell and McKirdy* have reported that oils rarely possess a Pr/Ph ratio of less than unity and that low values (<3) are associated with immature petroleum derived from terrestrial organic matter¹²⁶. Their analysis¹²⁶ of 55 Australian oils and condensates gives an average value for the Pr/Ph ratio of 4.8, while Tissot (unpublished results) obtained an average ratio of 1.35 from a much larger statistical survey (>1,000 oils). However, evaluation of the relative importance of oxic and anoxic conditions in oil-generating sedimentary environments requires a knowledge of the distribution of the individual values. Such an evaluation should also take into account the changes in oxicity/anoxicity of the palaeoceans, which have been deduced from geochemical evidence by Degens and Stoffers⁵⁰. (It seems that large areas of the ocean floor became anoxic as a result of ocean water stratification during several eras in the geological past, separated by oxygenated overturning.) Other factors which probably exert considerable influence on the Pr/Ph ratio of both oils and ancient sediments are the temperatures to which they have been subjected during diagenesis-maturation and their exposure to microbial activity.

Diagenesis, maturation and microbial activity

The effects of diagenetic and maturation processes on sediment biolipids can be investigated in two ways; first, by studying a uniform sediment which has been subjected to varying degrees of thermal treatment at different depths in the Earth's crust (for example, the Toarcian shales of the Paris Basin⁵), and second, by heating a sediment in the laboratory, simulating the natural environment. Both these methods have demonstrated that maturational processes can cause a decrease in porphyrin concentration due to degradation of the macrocycles and effect the conversion of DPEPs into etioporphyrins¹²⁷. Maturation also produces an initial increase in the isoprenoid concentration (relative to total organic carbon) in shales up to an evolutionary stage corresponding to 'the principal zone of oil formation'¹²⁸, followed by a gradual decrease¹²⁹ which parallels the changes in *n*-alkane content¹⁹. This suggests that the isoprenoid chains, like the *n*-alkyl chains, are attached to the kerogen during initial diagenesis and only liberated as hydrocarbons on thermal maturation.

The changes in geolipid content with depth have been published for two uniform sedimentary sequences—the Toarcian shales of the Paris Basin⁵ and the argillites of the Douala Basin, Cameroon¹²⁹. The Early Toarcian sediments were laid down in a marine environment with a high autochthonous and low allochthonous input^{5,130}. The Logbaba series of the Douala Basin consists of silty shales deposited during the Upper Cretaceous in a shallow marine deltaic environment¹³⁰. The oxicity/anoxicity conditions of deposition have not been reported for either sedimentary series. The less thermally mature shales of the Paris Basin have a Pr/Ph ratio of 0.5 (ref. 5), which suggests that the Toarcian sedimentary sequence was deposited in an anoxic palaeoenvironment. However, the Pr/Ph ratio increases with depth to a value of 1.3 for the deepest samples⁵. Therefore, mature shales of anoxic origin may be characterised by Pr/Ph values slightly greater than unity. An immature sample from a shallow depth (1,200 m) in the Douala Basin possesses a Pr/Ph ratio of 2.1 (ref. 129), indicative of oxic conditions of sedimentation. With increasing depth, and hence rock maturity, the Pr/Ph

value first increases to 4.9 at 'the principal zone of oil formation'¹²⁸, and then decreases to 1.5 (ref. 129), a change resembling that observed with increasing rank (maturation) for Australian coals⁷. This maturation trend therefore retains Pr/Ph values considerably greater than unity, which are representative of sediments deposited in oxic conditions. In laboratory heating experiments Connan¹³¹ has observed that thermal diagenesis favours preservation of pristane over phytane, so that the Pr/Ph ratio is expected to increase with maturation. A greater variety of sediment types and wider ranges of heating temperatures and times are required to fully evaluate this process. These limited results lead to the inference that maturation processes do not radically affect the Pr/Ph ratio to the extent that original palaeoenvironmental information is obscured. Hence, on an interim basis, other Pr/Ph data for ancient sediments and oils, allowing for the influence of maturation, can be taken as being valid.

Microbial effects present an additional factor. Both aerobic and anaerobic sulphate-reducing bacteria are capable of oxidising and destroying petroleum hydrocarbons¹³², and, although pristane and phytane are usually more resistant to microbial degradation than *n*-alkanes¹³³, they may also be metabolised¹³⁴, thereby affecting the Pr/Ph ratio¹³⁵. Microbial degradation of oils may therefore modify palaeoenvironmental information.

Conclusions

The oxicity or anoxicity of the environment of sedimentation exerts a marked influence, not only on the amount of organic matter preserved in a sediment, but also on its composition at the molecular level. This control over the extent and nature of the alteration of the organic matter occurs throughout the stages of sedimentation, namely (1) in the euphotic zone due to recycling of carbon, (2) during settling through the water column,

and (3) in the upper few centimetres of the bottom sediments due to microbial degradation. The limited amount of data presented here shows that the assignment of palaeoenvironment on the basis of chlorin or porphyrin content and the pristane to phytane ratio agrees with the indications of other data, such as those given in Table 3. Organic compounds may, therefore, display abundance patterns characteristic of their conditions of deposition, which can survive subsequent diagenesis and act as palaeoenvironmental indicators.

We wish to emphasise that we do not regard the Pr/Ph ratio as a single, definitive indicator of the sedimentation conditions of palaeoenvironments. Indeed, it is likely to become less definitive as additional data are acquired, since the number of processes and conditions which bear on this ratio is considerable. Some of these effects will need to be quantitatively assessed in order to make good use of Pr/Ph data. Furthermore, the amounts of other organic compounds and their relative ratios may also emerge as valuable palaeoenvironmental indicators. For example, there is every indication that perylene is one such compound^{148,149}. Even labile lipids, such as carotenoids, may be expected to help assign palaeoenvironmental conditions of sedimentation when their diagenetic pathways are more fully understood.

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Table 3 Characteristics of oxic and anoxic sedimentation

	Sedimentation conditions		
	Oxic	Anoxic	Refs
Physical characteristics of environment			
O ₂ in bottom waters	Yes	No	<i>a priori</i>
H ₂ S in bottom waters	No	Yes	<i>a priori</i>
Water column circulation	Complete	Restricted	62
Current energy	High	Low	63
Sedimentation rate	Low	High	63
Sediment grain size	Coarse	Fine	136
Bottom fauna	Present	Absent	137
Redox potential	Positive	Negative	138
Chemical characteristics of sediments			
Mo content	Low	High	137, 138
U content	Low	High	139
Co, Cu, and Pb content	Low	High	137, 138
Rb content	High	Low	137, 138
NH ₃ concentration	Low	High	140
P ₂ O ₅ content	Low	High	137, 138
PO ₄ ³⁻ concentration in pore waters	Low	High	50, 140
% Non-exchangeable Mg/% Montmorillonite	Low	High	141, 142
Total S content	Low	High	137
Br content	Low	High	137, 138
I content	High	Low	137, 138
I ⁻ /IO ₃ ⁻ ratio	<1	>1	143
I/C ratio	High	Low	138, 144
Dissolved gases	Low	High	50
Organic C content	Low	High	49
% HCl-extractable sugars released by EDTA	55–75	25–45	55
Rate of disappearance of amino acids	High	Low	145–147
Chlorin content	Low	High	Table 1
Pr/Ph ratio	>1	<1	Table 1

This comparison of data for these two depositional environments is designed to indicate their differences only on a relative basis. For example, I/C ratios for both environments are low in absolute terms (considerably less than unity^{138,144}), but markedly higher values are consistently observed in oxic, compared to anoxic, sediments.

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Computer simulation of the solvent structure around biological macromolecules

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The structure and energetics of the solvent, both ordered and disordered, in a small peptide crystal and in the triclinic lysozyme crystal have been simulated using the Monte Carlo method. The results are in good agreement with the experimental data and provide insight into the role of solvent structure around biological macromolecules in solution.

THE solvent system which makes up the environment of biological macromolecules has a central role in their structure and function¹. In spite of this crucial importance, little is known

about the structure and properties of such systems, either from an experimental or theoretical viewpoint^{2,3}. This is because of the inherent difficulties associated with the study of the liquid state in general and liquid water in particular^{4,5}. Experimental work³ using preferential ion binding, isopiestic measurements, light scattering, NMR, infrared and diffusion studies all indicate that there is a layer of water surrounding macromolecules in solution which is phenomenologically distinct from bulk water, although the quantity and nature of this structure varies greatly with the technique used. Crystallographic studies of globular proteins have become an important tool in this area. Protein molecules in crystals, although them-

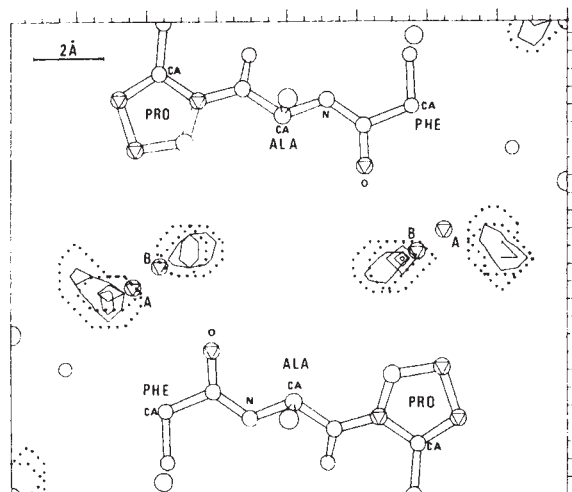


Fig. 1 Superposition of successive sections (0.38 Å apart) of the probability map of water molecule positions in the cyclic peptide crystal for the Stillinger potential. A and B represent the observed X-ray water positions of two symmetry related waters each with two alternative positions 1 Å apart. The calculated probability of finding a water molecule density in a given volume (0.081 Å³) is indicated by contours of constant probability, with the zero contour dotted (probabilities are contoured in increments of 0.05). Peptide atoms within 2 Å of the sections are shown with those near the section height distinguished by triangles.

selves compact, form rather open arrays. These arrays contain relatively few close contacts between protein molecules and incorporate a large amount of solvent (26–65% by volume⁶). Thus the molecules may be considered to be in a very concentrated solution rather than in the solid state. Improvement in refinement methods for such structures^{7,8} has begun to reveal an ordered water shell around the protein molecules. The extent of this shell is still somewhat controversial, but is generally considered to be confined to water molecules in direct contact with the protein. Measurements of the diffusion of small molecules through protein crystals⁹ and of the volume of solvent replaceable by salt ions¹⁰ confirm that large parts of the solvent in the crystals behave in a way similar to bulk water. These crystals, then, are excellent models for studying the interplay between macromolecules and their solvent.

Method

In this study we extend the Monte Carlo method to the simulation of the water structure around molecules of biological interest in crystals and in solution. The method is illustrated with applications to two crystals, one of a small cyclic peptide which serves as a test system, and the other of a protein. The Monte Carlo method and the related method of molecular dynamics have been very successful in reproducing the thermodynamic and structural properties of bulk water^{11,12} and the water structure around an isolated ion in solution¹³. In these methods enough states of the solvent in the system are obtained to allow the calculation of the desired macroscopic properties by weighted averaging over the states¹⁴. The desired statistical average of any property X is given by

$$\langle X \rangle = \frac{[\sum_i X \exp(-E_i/kT)]}{[\sum_i \exp(-E_i/kT)]}$$

taken over a 'representative' set of states i . For 'dense' systems such as water, the selection of states in the Monte Carlo method is by the Metropolis algorithm¹⁴ in which small perturbations of the system relate one state to the next in a Markov chain process. The method is in principle exact (*ab initio*) and generates the statistically averaged configurational properties on convergence¹⁴. As all water–water and water–protein interactions are represented explicitly, the accuracy of the description of the solvent structure—both ordered and disordered—is then limited only by the reliability of the model and potentials used.

To apply this method to the simulation of solvent structure

around protein molecules, several problems must be considered. First, a fairly good model of the protein structure itself must be available. The water structure resulting from the simulation will be sensitive to the position of surface groups on the protein and these are precisely the ones normally least well determined in the X-ray work. In many structures several of these side chains are disordered and such disorder would need to be included in the simulation. Second, as charges on ionised side chains and ordered salt ions often dominate the forces involved in their vicinity, it is important that an adequate model of their distribution be available. Furthermore, if a large concentration of salt ions is present in solution their inclusion in the simulation would be required. Third, there is a limit of about 500 on the number of water molecules that can be included explicitly in the simulation, set by the power of currently available computers. Thus, if dilute solution conditions are being simulated, waters far from the protein molecule must be represented by a reaction field¹⁵, whilst in a crystal environment the number of waters per asymmetric unit generally should not exceed this limit. Most protein crystals have substantially more water molecules than this. These three conditions of good model, low salt and small size, place a considerable restriction on the choice of system. A fourth consideration is the choice of potentials to be used in calculating the energies. For the protein portion of the system several sets of parameters are available and their range of reliability in crystals of small model compounds is relatively well established^{16,17}. For water several alternative models are currently in use and have been applied to the pure liquid^{11,12}. It should be noted that no study has been made of water in biological systems using these potentials and that their adequacy for this purpose is one of things we direct our attention to here^{28,29}.

Once a suitable system has been found and potentials decided on, we proceed as follows. A starting configuration is constructed by packing water molecules around the solute molecule in an arbitrary array to give a density equal to that of bulk water or, if available, the observed number of water molecules per asymmetric unit of a crystal. Individual water molecules are then chosen at random and moved a random fraction of a maximum translation, usually 0.25 Å. An axis of rotation is chosen at random (x, y or z) and the molecule rotated by a random fraction of a maximum rotation (10°). The change

Table 1 Comparison of the simulated and experimental ordered water positions in the cyclic peptide crystal for two different water models

Water molecule	Rowlinson water model		Stillinger water model		X-ray R.m.s. vibration
	Deviation from X-ray position	R.m.s. movement during simulation	Deviation from X-ray position	R.m.s. movement during simulation	
On two-fold axis	0.6	0.72	0.8	0.71	0.49
	—	—	—	—	0.49
In a general position	0.5	0.52	0.2	0.58	0.45
	0.5	0.50	0.4	0.50	0.45
	0.4	0.56	0.6	0.69	0.45
	0.4	0.52	0.5	0.54	0.45

The r.m.s. movements are about the average positions of the water molecules and, for the simulation, are relative to stationary peptide molecules. The units of all quantities given here are Å. Inclusion of the peptide movement is difficult because of the unknown correlation between peptide and water vibrations, but would be expected to increase the values. Dashes for the second water indicate failure to reproduce this feature. Averages are based on ~140,000 configurations after convergence.

Note the molecules are not constrained by symmetry relations and thus comparisons are given for each fully ordered molecule in the unit cell.

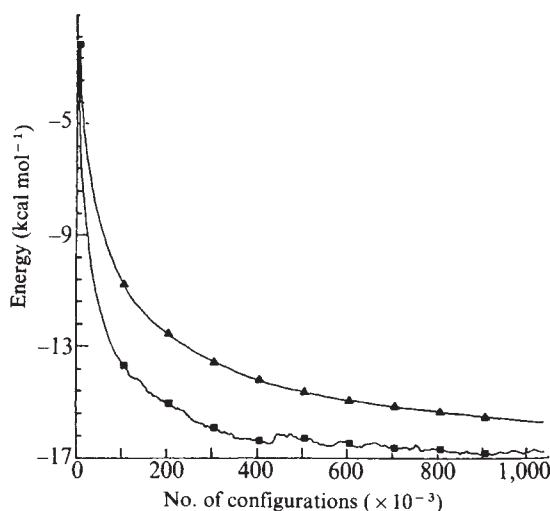


Fig. 2 The average energy of the water of hydration of the triclinic lysozyme crystal as a function of the number of configurations generated in the Monte Carlo simulation. The upper curve (▲) corresponds to the cumulative statistical average, while the lower curve (■) gives the statistical average over sequential sets of 5,000 configurations.

in energy of the system (consisting of the unit cell contents interacting with themselves and those other translationally related atoms in the range of the potentials used) is calculated. If this change in energy is favourable (more negative) the new configuration of the system so generated is accepted. If the change increases the energy of the system the Boltzmann factor of the energy change $\exp(-\Delta E/kT)$ is compared with a random number between 0 and 1. If this factor is greater than the random number the move is accepted, otherwise it is rejected and the old configuration is counted again in the configurational average. This is the Metropolis algorithm¹⁴ and can be shown to generate configurations with a probability $\exp(-E/kT)$. It then follows that the required configurational averages are simply the arithmetic average over the Boltzmann weighted configurations. The initial configurations generated are used to 'move' the system away from the arbitrary starting situation and then subsequent configurations are used to calculate desired properties.

The properties we calculate fall into two classes: structural and energetic. Structural properties, such as the mean positions and temperature factors of the ordered waters, and the probability of finding a water molecule at a given position in the unit cell, are most useful for relating the results to X-ray experiments and for an appreciation of the degree of order in different parts of the system. Energetic properties, such as the average energy and energy distributions of waters in different environments, are appropriate for understanding the role of water in stabilising the system.

Hydration in the cyclo- (L-Ala-L-Pro-D-Phe)₂ crystal

As a test of the method we have simulated the water structure in a small and well defined system, crystals of the hexapeptide cyclo-(L-Ala-L-Pro-D-Phe)₂, for which a precise structure is available from X-ray work¹⁸. These crystals contain two hexapeptides per unit cell, in space group P2₁2₁2, with the twofold axis relating two halves of the peptide. The structure is unusual in that all interpeptide contacts are mediated by water molecules. There are four fully ordered waters in general positions in the unit cell (1 per asymmetric unit), two fully ordered on the twofold axes (1/2 per asymmetric unit) and four other molecules each identified as having two alternative positions in the asymmetric unit 1 Å apart. Two others were positioned on the twofold axes in the crystallographic refinement in the centre of apparently disordered regions and a

further four could not be located because of their disorder. Thus this system, in addition to its intrinsic interest, provides a benchmark for the simulation method.

We have simulated the solvent structure of the unit cell of this crystal using two different water models (those of Rowlinson¹⁹ and Stillinger and Rahman¹²) and potentials for the solute derived from small crystal studies (A.T.H., E. Huler & S. Lifson^{16,17}). From a statistical average of the positions of water molecules during the simulation we are able to compare positions and thermal motion with the X-ray data for the six fully ordered waters in the unit cell (no intra-unit cell symmetry is included explicitly in the simulation). Table 1 shows this comparison. Both potentials reproduce these ordered positions quite well to accuracies of around 0.5 Å, with the exception of one molecule on the twofold axis. The degree of thermal motion is also reproduced well.

Reproduction of the less ordered water structure is illustrated in Fig. 1 which shows sections of the probability map of water positions for regions in the unit cell containing the water with two alternative positions. A most satisfactory agreement is found between the calculated peaks from the simulation and the observed alternate water positions in this region. A similar map for the disordered region shows low probability contours representing more disordered waters. Thus all levels of order and position in this crystal are produced with a good degree of fidelity and we feel confident in applying the method to a more complicated, but biologically more relevant, situation. A detailed description of this work is given elsewhere (A.T.H., J. M. & D. J. Osguthorpe, in preparation).

Simulation of the solvent structure in a protein crystal

We have used triclinic crystals of hen egg white lysozyme, which satisfy fairly well the conditions specified above. The crystals are grown from 2% sodium nitrate and 50 mM acetate buffer²⁰. Work so far has been based on an X-ray model at 2.5-Å resolution with an *R* factor of 35% (ref. 21), but an improved model with an *R* factor of 26% is now available (J. L. Sussman, O. Herzberg, J. M. & A. Yonat, in preparation), and a very high resolution X-ray study²² is in progress. Comparison with the same protein molecule in a different crystal form²³ shows that the structure is very similar²¹ and therefore likely to be representative of the conformation in solution. The positions of nearly all side chains are fixed and the positions of around 80 ordered water molecules and eight nitrate ions have been identified crystallographically (J. M. & A. Yonat, in preparation). From extensive titration data²⁴ 28 charges can be identified on the surface of the protein with a net charge of +8 at this pH (4.5), so that the eight identified nitrate ions neutralise the system. A determination of the sodium content of the crystal by atomic absorption spectroscopy gives a value of 0.5 ± 0.2 sodium atoms per unit cell, while measurement of the volume replaceable by salt ions would suggest a value of about one sodium and one nitrate disordered per unit cell (J. M. & A. Yonat, in preparation). The system to be simulated thus consists of 303 water molecules, eight ordered nitrate ions and 1,001 non-hydrogen atoms in the P1 unit cell. 623,000 configurations of the waters were generated to randomise the system and properties were calculated from a further 400,000 after convergence. The energy convergence is shown in Fig. 2. The Rowlinson water model¹⁹ was used here, while the potentials for the protein were based on those derived by A.T.H., E. Huler and S. Lifson^{16,17}.

Description of the solvent structure

Several properties of the system illustrate the role of water in it. Figure 3 shows a plot of the average root mean square deviation of molecules from their mean positions as a function of the intimacy of their interaction with protein molecules. Most strikingly, molecules far from the protein move around over larger distances than those near to it, and there is a gradual change in average ordering, from highly ordered near the

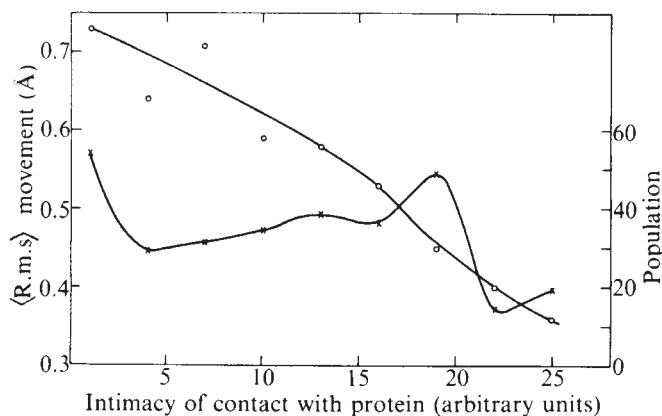


Fig. 3 (○), Average root mean square movement of waters about their mean positions as a function of intimacy of contact with protein molecules. The units of contact are calculated as

$$\frac{80 \cdot \sum 1/r^2}{1} + \frac{30 \cdot \sum 1/r^2}{2} + \frac{20 \cdot \sum 1/r^2}{3}$$

where sum 1 is over distances to charges, sum 2 over distances to polar atoms and sum 3 over distances to nonpolar atoms. This expression may be regarded as a weighted sum of contacts with the protein: the larger the number, the more and/or stronger the interactions. (×), No. of water molecules in each interaction range.

protein to much less ordered far away. Figure 3 also shows the number of water molecules in each of these ranges of contact with the protein. Closer inspection shows some variation with respect to the general picture. It is usual to refer to all waters within say, 3.5 Å of the protein as a first hydration shell, and to consider their properties as being similar. Inspection of contacts shows that over half the molecules in the cell would fit this definition of first hydration shell, but their r.m.s. movements indicate their degree of ordering varies widely. This arises from the very different environments of waters within this layer and so some care should be used in employing this concept. Comparison of the mean positions of the most ordered waters with those observed in the X-ray map gives a

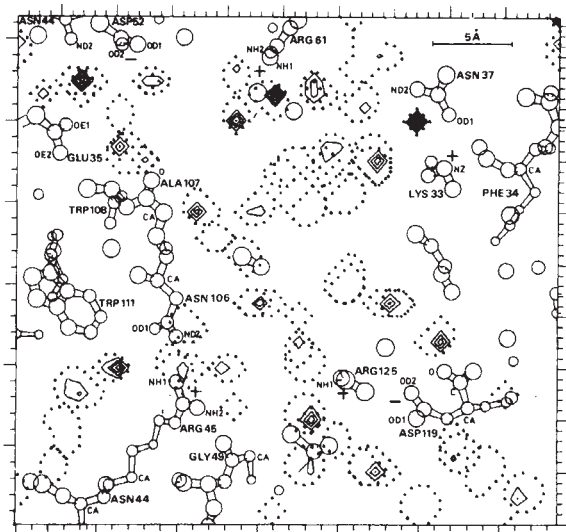
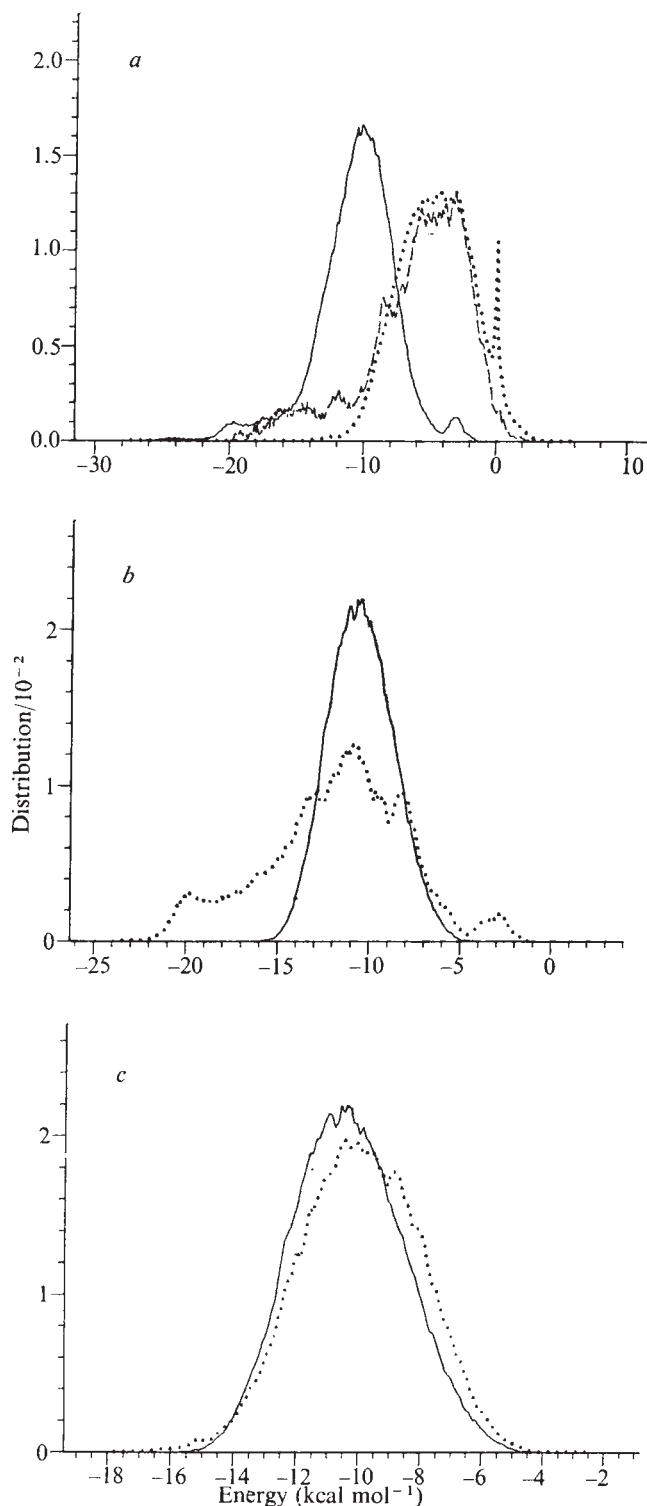


Fig. 4 Superposition of two sections of the probability map of water in the triclinic lysozyme unit cell separated by 0.78 Å. The sections pass through a solvent filled cavity between neighbouring protein molecules. The probability, corresponding to finding a water molecule in a volume element of 0.438 Å^3 , is contoured in increments of 0.1. Zero contours are dotted. The compact, high density features represent ordered water molecules, the smaller the feature and the more contours, the more ordered. In the top left-hand corner are the carboxyl groups of the two active site residues Glu 35 and Asp 52, with an ordered water between them. Other ordered waters are all near protein groups and almost always near charges. Further from the protein molecule surfaces the waters are seen to be much less ordered.

r.m.s. deviation of around 1.2 Å. A comparison of contacts with protein atoms up to 4.2 Å away for each water shows 49 of the 80 X-ray waters to occupy the same environmental niches as the simulated ones. Figure 4 shows two sections of the probability map of the waters in a section of the unit cell containing a relatively large cavity. Near the protein molecules defining the cavity, and particularly near charges, sharp peaks are seen identifying highly ordered waters, while in the centre

Fig. 5 Energy distributions of waters in different environments in the triclinic lysozyme unit cell. *a*, All waters, total energy (—) and energy partitioned into water–water (....), and water–protein (---) contribution. *b*, Waters in close contact with the protein (....) (see Fig. 3 for definition of proximity) and water in bulk water (—). *c*, Water far from the protein (....) and water in bulk water (—).



much lower probabilities are apparent, representing essentially disordered waters.

Figure 5a shows the overall energy distribution for the waters in the unit cell. Three curves are shown, total energy, water-protein energy and water-water energy. The energy is fairly evenly divided between these two components, with some interesting irregularities. In the water-water curve there is a sharp peak at zero energy representing water almost completely isolated from the others in the system. Examination of the contacts of individual waters with other elements of the system shows there are nine of these. Eight of them are buried in a single protein molecule, two of them together, the others separately, and one is isolated by inter-protein molecule boundaries. This molecule and four of the other buried ones do not have energetically very favourable environments, and produce the small peak at high energy in the total energy distribution. The water-protein curve exhibits a long tail at low energies representing water tightly bound to the protein, closely corresponding to the most highly ordered molecules, and often in contact with one or more charges³. Figure 5b shows a comparison of the energy distribution for the 78 waters in closest contact with the protein (by the same criterion as used for the r.m.s. deviation analysis), and the energy distribution of water in bulk water for the same water model (obtained by a reproduction of the simulation of Barker and Watts of 64 water molecules in a cube¹¹). The differences in these distributions reflect the different nature of the water structure adjacent to the protein and that of bulk water. The proportion of the area in the low energy tail is, as one would expect, much higher than in Fig. 5a. In contrast to this, Fig. 5c shows a comparison of the energy distribution of the 51 waters more than 4.2 Å from any protein atom with the same bulk water distribution. By this measure, these waters are clearly very similar to bulk water³, and only slightly constrained by their more ordered neighbours. It should be emphasised, however, that most of the waters in the crystal are intermediate between the two distributions shown in these two figures and show an intermediate degree of order. Many of the waters close to the protein are considerably lower in energy than those of bulk water. Thus water in channels between protein molecules separated by two or three water molecule diameters may be in a lower energy state than bulk water and so act as a 'long range force' between the protein molecules. Water acting as a 'glue' in this way may be of general importance in biological self-assembly processes where a high degree of interaction between neighbouring molecules is not essential, but proximity is. The effect would be important for surfaces between particles separated by up to 6 or 7 Å. In this argument we have ignored the change of free energy arising from decreased entropy of ordering. Methods for calculating the entropy from the Metropolis algorithm are currently being developed^{25,26}, but in any case the effect on this argument will not be large. At most the

free energy from increased ordering can be no more than that of freezing bulk water (about 1.5 kcal mol⁻¹ at 300K (ref. 5) and this has to be compared with an enthalpy change of 5–10 kcal mol⁻¹ for the most ordered waters (Fig 5b).

These results are preliminary, and together with those on the cyclic peptide crystal demonstrate the power of the method. We have yet to examine in detail the water structure around ions, exposed hydrophobic groups and in the narrow channels between protein molecules. The approach is easily extendable to related systems. In particular, substrate related molecules can be introduced into the crystal²⁷ and their effect on the water structure examined. The simulation can be performed for a protein molecule in dilute solution and for an unfolded chain in solution, and the changes in water structures, and energetics of hydration on folding can be compared.

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Note added in proof: Clementi *et al.*²⁸ have recently computed the interaction of a single water with the lysozyme molecule, using potential functions derived from *ab initio* calculations of water amino acid interactions.

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Molecular morphology in semicrystalline polymers

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The rapidity of crystal growth from undercooled polymer melts precludes transformation of the randomly coiled macromolecules to the regularly folded morphology generally ascribed to semicrystalline polymers. The topology of the copiously interspersed chains in the liquid state must be largely conserved during the process of crystallisation, pre-existing entanglements being concentrated in the non-crystalline regions.

THE prevalence of a lamellar morphology in semicrystalline polymers is well established. This morphology is manifested not only in the 'single crystal' platelets that separate from a dilute solution, but also in polymers that crystallise from the quiescent melt. Typically, the lamellae in melt-crystallised polymers are 100 to 500 Å in thickness¹; their breadths may be measured in μm. The surface of the lamella is a 001 lattice plane, that is, it is transverse to the rectilinear axis of the chain in the conformation that is characteristic of the crystalline form. Amorphous polymer is interspersed between the lamellae. It may comprise a substantial

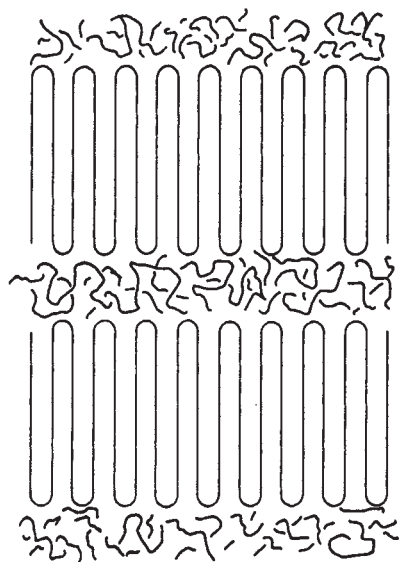


Fig. 1 The regularly folded model for a semicrystalline polymer. The amorphous interlayers are represented without commitment on the interconnections between the two phases.

fraction of the material, sometimes in excess of half of the semicrystalline polymer¹. According to its infrared spectrum, density, glass transition and other properties, the material comprising the inter-lamellar layers closely matches the bulk amorphous polymer in all respects^{2,3}.

This much of the description of the morphology of polymers crystallised from the melt rests on unassailable evidence. In contrast, the arrangement of the molecular chains within this system of crystalline lamellae and intercrystalline amorphous regions has long been disputed. The molecular chains, being tens or hundreds of times the length required for a single passage through a crystal lamella, obviously must traverse one or more lamellae many times. The model that has gained widespread acceptance depicts each polymer molecule as regularly folded like the wool in a woven fabric^{2,3}. On emerging from the face of a crystalline lamella the chain executes a reversal of direction and re-enters at once the same lamella at a location immediately adjacent to its preceding passage, according to this model. Traversal of the lamella is assumed to repeat in this fashion, always with adjacent re-entry, for the full length of the molecule.

This regularly folded description of the molecular morphology in a polymer allowed to crystallise from its melt is depicted in Fig. 1. Interconnections between crystalline lamellae and the amorphous interlayers, whose presence is undeniable, are deliberately omitted, as this is a feature not definitively resolved by advocates of the model.

The regularly folded model briefly described has enjoyed widespread appeal. It is simple and pictorial. The obscurities of random chains and the statistical abstractions required for comprehending them are conveniently avoided by its adoption. The model was threatened some years ago when it first became apparent that a substantial fraction of amorphous material occurs as a separate phase situated between the lamellae. Moreover, physical and chemical properties indicate firm bonding of one lamella to its neighbours. Clever inventions such as 'tie chains' between lamellae were conceived in order to sustain the model against criticisms on these grounds. Despite an abundance of contrary evidence from analysis of the density, thermodynamic properties and lamellar thickness in relation to molecular weight and temperature of crystallisation²³⁻²⁵, the regularly folded model has survived attacks for over 20 years. It remains the cornerstone of current explanations of physical properties, including plastic flow, of semicrystalline polymers.

Recent results of neutron scattering experiments⁴⁻⁷ that yield information on the dimensions and arrangement of a deuterated polymer dispersed in the protonated host [for example, deu-

teropolyethylenes, $(CD_2)_x$ dispersed in $(CH_2)_x$], the two being cocrystallised, provide fresh evidence that is irreconcilable with the regularly folded model⁸. Here we call attention to the constraints incident on the process of crystallisation of randomly coiled long chains. The circumstances under which crystallisation proceeds have an important bearing not only on the specific issue raised above, but also on the over-all topology of molecular chains in semicrystalline polymers. This latter is a matter of foremost relevance to the interpretation and understanding of the physical and mechanical properties of semicrystalline polymers.

The rate of crystal growth in polymer melts cooled below their melting points generally is very rapid, especially for typical degrees of supercooling which are on the order of 20 °C or more. That the rapidity of growth of the crystal lamellae is a factor of foremost pertinence to the molecular morphology of the semicrystalline polymer has been noted previously^{9,10}. The full implications of measured rates of growth have not, however, been examined in detail.

The edge of a growing crystal lamella is shown schematically in Fig. 2 in the plane of the lamella. The molecular chain from which a sequence is deposited at the 'kink' in the growing edge is also included in the diagram. The growth rate normal to the edge is indicated by G . The rate of development of the outermost layer by deposition of sequences at the kink is denoted by g .

Lindenmeyer *et al.*¹¹ and Hoffman *et al.*¹² have measured the radial growth rates of spherulites in linear polyethylene, or polymethylene (PM), as a function of the degree of supercooling. These rates may be identified with G . For a PM of molecular weight about 10^5 supercooled some 25 °C below the melting point $T_m^\circ \approx 146^\circ\text{C}$, $G \approx 1 \mu\text{m s}^{-1}$. (The thickness of the crystal lamella formed in the conditions stated is about 200 Å. The amorphous interlayer measures about 60 Å in thickness.) Similar rates G were reported previously for poly(decamethylene adipate) at comparable degrees of supercooling¹³. The radius of gyration of PM of this molecular weight is approximately 140 Å, or 10^{-6} cm (ref. 14). Hence, the domain originally occupied by a random coil in the melt is enveloped during an interval little greater than 10^{-2} s.

The estimated rate of deposition g at a 'kink' along the growing lamella is $50\text{--}500 \mu\text{m s}^{-1}$, or $10^5\text{--}10^6$ sequences s^{-1} according to the results in ref. 12 for multiple nucleation growth (regime II)¹⁵. The domain of a randomly coiled molecule is traversed by the kink in 5×10^{-5} to 5×10^{-4} s, according to these estimates.

The rate of relaxation of the PM molecule with $M = 10^5$ at 120 °C in the melt is on the order of 1 s as estimated from the theories of Rouse¹⁶ and of Bueche¹⁷. Approximately the same figure may be inferred from the rate of shear at which viscous flow becomes non-Newtonian¹⁸. Clearly, relaxation of the molecule as a whole is much too slow to occur within the time available during its envelopment by the advancing crystal front. In these circumstances, thermal motions of units of the chain can effect only limited rearrangements of the molecular conformation in the course of its active involvement in the crystallisation process.

The rate of relaxation of a sequence of units within the chain

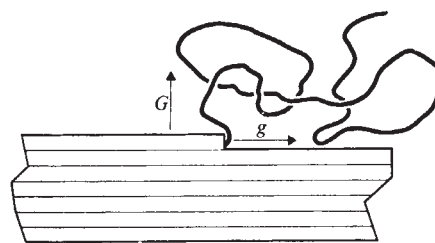


Fig. 2 Schematic representation of a lamellar crystallite in process of growth on a face (for example, 100 or 110) transverse to the chain axis. For an orthorhombic crystal such as polyethylene, the chain axes are normal to the diagram. Amorphous interlayers occur above and below the lamella. A random molecule involved in deposition of a sequence at a 'kink' in the growing edge is shown. Growth rates along the edge and normal to it are indicated by g and G , respectively.

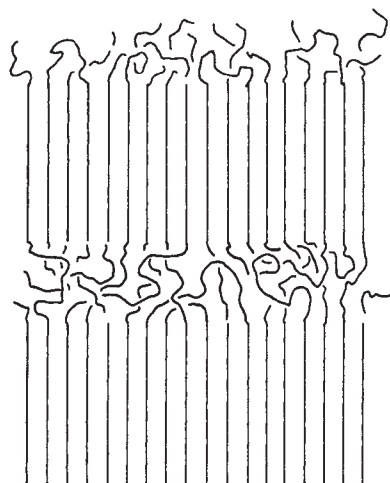


Fig. 3 Molecular arrangements in a lamellar semicrystalline polymer in which different molecules are extensively intertwined. Re-entry of a given lamella, although of frequent occurrence, is not generally at the site adjacent to its emergence from the crystal lamella.

increases rapidly with decrease in the length of the sequence. For sequences of 100–200 C–C bonds in a PM chain, corresponding to one traversal of a crystal lamella formed in the conditions stipulated above, rates of relaxation are estimated to be comparable to those estimated for g expressed in number of sequences per unit time. Thus, the conformational relaxation rate seems to be sufficient to support the observed rate of deposition of sequences.

A much greater time (> 1 s) would be required for the complete rearrangement of the molecule having a length 50–100 times that of a sequence, and for its disengagement from entanglements with other molecules. Completion of these processes would be essential for deposition of the molecule in regularly folded array. That this could be accomplished within the interval during which a molecule is incorporated in the crystal is inconceivable. Instead, the growing crystal edge must feed on whatever chains are suitably situated and hence available for deposition at the instant when the growth front meets a small element of the untransformed liquid. Sequences from various molecules are therefore selected for deposition in the growth layer. The process thus conceived places minimum demands on conformational rearrangements of the sections of a contributing molecule other than the sequence that undergoes deposition.

The morphology that is envisaged on the basis of these considerations is represented in Fig. 3^{9,19}. Successive sequences in a given layer of the crystal lamella are contributed by a plurality of molecules, instead of by only one, as the regularly folded model requires. Return of a given chain to the lamella from which it emerges must occur frequently, as is indicated in Fig. 3, for reasons that are implicit in the randomness of the chains in the amorphous phase on the one hand and restrictions on volume on the other (see ref. 9). Return at the site adjacent to its emergence must be comparatively rare, however. Elementary statistical considerations argue compellingly for random re-entry. Moreover, traversal of the amorphous interlayer must frequently occur followed by entry of the adjacent lamella as is indicated in Fig. 3.

It should be noted also that the first sequences of a molecule to undergo crystallisation may be remote from one another along the length of the molecule. These sequences may enter the same or different layers at the edge of the lamella. This would be more clearly evident from Fig. 2 if the full length of the molecule were shown to scale. A given molecule becomes affixed to the crystal by incorporation of several sequences, distributed over the length of the molecule, before a major fraction of that molecule undergoes crystallisation. Hence, the preponderance of the sequences acquired by the crystalline layers are contributed by molecules that already have become engaged in crystal lamellae. Over-all rearrangement of such molecules cannot therefore occur. This restriction must be operative even in conditions such that crystal

growth is comparatively slow, for example, at low degrees of supercooling. Regardless of details of mechanism, the gross conformational changes that would be required to disentangle the molecules from their neighbours (an utter necessity for regular folding!) are precluded.

Recent results of neutron scattering experiments on semicrystalline polyethylene containing a small portion of deuterated polyethylene^{4,6,7}, and on isotactic polypropylene⁵ in which the deuterated analogue is similarly incorporated are confirmatory of the foregoing conclusions. These results may be stated succinctly as follows: (1) the radius of gyration of the deuterated species in the semicrystalline polymer is the same as in the melt, within limits of experimental accuracy; (2) the scattering function at larger angles is incompatible with regular folding of the deuterated molecules, or with any other kind of folding such that re-entry occurs predominantly in adjacency to the preceding sequence^{20,21}. Detailed analysis of the scattering function leads to the conclusion that adjacent re-entry occurs infrequently^{20,21}. It shows further that the predominant modes whereby a molecule successively traverses lamellae consist of (i) re-entry of the same crystal layer at a point removed from its previous passage and, (ii) penetration of the amorphous interlayer followed by entry of the neighbouring lamella. These modes are illustrated in Fig. 3.

Regular folding of the chain with adjacent re-entry throughout its entirety would be incompatible with observation (1), as Schelten *et al.*⁴ have pointed out. The result (1) is confirmatory, however, of the conclusion above that the gross spatial configuration of the chain may undergo little alteration during the brief interval of its envelopment by the advancing crystal front. Both the slow relaxation of the molecule and the restrictions imposed by deposition of the first sequences to be incorporated in lamellae may be responsible for this revelation of neutron scattering experiments.

According to opinions currently advocated by adherents to the regularly folded model, the results cited above are peculiar to specimens that are rapidly crystallised, such as those used in neutron scattering studies. In the case of polyethylene, rapid crystallisation seems to be required in order to achieve molecular dispersion of the deuterated polymer. By implication, regular folding may be expected to prevail in slowly crystallised samples, as if this should be the mode of preference in circumstances such that kinetic factors are no longer controlling. Against this view, it must be noted that the presumed preference for regular folding is unsupported. In fact, the conformations required for regular folding are of higher energy than the average for the random chain; regular folds are statistically disfavoured as well. On the experimental side, evidence for regular folding is as lacking for slowly crystallised specimens²⁴ as for those that are rapidly crystallised, such as the ones used in most of the neutron scattering experiments conducted to date. If acquisition of sequences by the crystal lamellae from the copiously entangled molecules of the melt occurs at random, in the manner inferred above, then regular folding is untenable regardless of the rate at which crystallisation is conducted. On the same basis, the spatial domain a molecule is destined to pervade in the semicrystalline polymer is fixed, approximately at least, when only a small fraction of the molecule has been engaged in the crystal lamellae. Hence, the radius of gyration should be little affected by crystallisation at low as well as at high rates.

An important corollary of the above conclusions concerns the topology of the system of molecular chains in a semicrystalline polymer. The arguments above, and their confirmation by the radii of gyration found through neutron scattering, lead at once to the assertion that the state of the interspersions of chains prevalent in the amorphous state must be conserved in the process of crystallisation. In other words, the topological relationships of the chains to one another in the semicrystalline polymer must approximately duplicate those that existed previously in the melt. It is well established that polymer chains assume randomly coiled configurations in the melt²². The comparatively large domain pervaded by a given molecule is shared with a hundred or more other molecules, similarly configured in voluminous random

coils. Hence, the molecules are profusely intertwined, or entangled. These entanglements are excluded from the crystal lamellae, except for those that may occasionally be incorporated as defects. Since the entanglements cannot be dissipated during crystallisation for the reasons stated, they must be concentrated in the amorphous regions.

One is thus forced to conclude that neighbouring crystal lamellae are profusely interconnected. Connections are provided not only by chains emanant from one lamella that enter the other, but also by the entanglements involving chains that re-enter the same crystalline layer after passage through a portion of the adjoining amorphous layer.

These considerations of the molecular morphology in semi-crystalline polymers lead at once to the conclusion that large, irreversible deformations, such as occur in plastic flow or cold drawing, must entail either (1) rupture of molecular chains or (2) destruction of pre-existing crystalline regions, that is, melting. The copious connections between lamellae preclude their movement relative to one another without disruption either of the chemical structure or of the intermolecular morphology. Inasmuch as plastic flow is not accompanied by appreciable decrease in molecular weight of the polymer, alternative (1) may be dismissed. Melting of crystalline regions in response to local stresses is therefore implicated unambiguously. The melting of a small crystalline region thus induced normally will be followed immediately by recrystallisation, a different array of sequences being incorporated in the regenerated crystallite in a pattern compliant with the prevailing stress.

According to current views plastic deformation of semi-crystalline polymers is considered to involve sliding or tilting of lamellae, their integrity and that of the molecules involved in them

being preserved in the course of the process. The experimental observations briefly cited above and the deductions following directly from them show such mechanisms to be altogether untenable. Drastic revision of prevailing ideas on the processes occurring at the molecular level in plastic deformation of semi-crystalline polymers seems therefore to be essential if progress is to be made.

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letters to nature

Origin of slow moving object Kowal

SLOW moving object Kowal^{1-3,5}, now named Chiron^{4,6}, is generally agreed to be a new asteroid, although Gingerich⁴ and Ashbrook⁶ have suggested that it might be a 'deep-frozen' comet. Its orbit lies mainly⁵ between Saturn and Uranus but what is an asteroid doing so far from the main belt between Mars and Jupiter? If Chiron was born in its present orbit and is part of a new asteroid belt, then its existence may put constraints on models of the origin of the Solar System (note that a belt between Saturn and Uranus would violate Bode's law^{7,8}). It may be simpler, however, to look for a more recent origin. One possibility, which is examined here, is that Chiron has been flung into its present orbit by a sort of gravitational sling-shot, having been successively perturbed, or even temporarily captured, by Jupiter and Saturn.

This suggestion stems from calculations by Hunter^{9,10}, who investigated the stability of satellites of Jupiter and concluded, from many numerical orbital calculations, that some asteroids could be ejected satellites and that asteroids could be captured from the main belt and that some might then be ejected into orbits between Jupiter and Saturn. These calculations strongly suggested¹⁰ the existence of a belt between Jupiter and Saturn of asteroids with apparent magnitudes fainter than 15. As far as I know, no systematic search has been made for such a belt.

If it were to exist, then, because the ratio of the radius of the sphere of influence of Saturn to the distance between the orbits of Saturn and Jupiter is about the same¹¹ (8.5%) as the ratio of the sphere of influence of Jupiter to the distance between the orbits of Jupiter and Mars, it seems reasonable that Saturn might equally

efficiently capture some of the outer asteroids in the belt and subsequently eject some again into orbits between Saturn and Uranus. As asteroids shine by reflected light, their brightness follows roughly¹⁰ an inverse fourth power law. A typical such asteroid with an orbital radius of 18 AU. (Chiron is at present 17.8 AU from the Sun) would be 9.1 mag fainter at opposition than the same asteroid in the main belt (median semi-major axis 2.7 AU). A typical bright asteroid has an apparent magnitude¹² of about 10 and so Chiron, which is not far from opposition¹ and has a visual magnitude between 18 and 19 (ref. 2), would be a bright member of such a trans-Saturnian belt.

The orbital eccentricity (0.38) and inclination (7°) of Chiron⁵ are also consistent with its having been captured and ejected by Jupiter and Saturn, as they are comparable with those of asteroids in the main belt and Hunter's calculations¹⁰ suggest that ejected satellites also have eccentricities and inclinations comparable with those of normal asteroids. Furthermore, because Chiron actually comes inside the orbit of Saturn at perihelion, there are still relatively close encounters between Chiron and Saturn (1.1-1.3 AU according to Marsden⁶) and any ejection by Saturn seems likely to have occurred in the astronomically recent past.

It would be very interesting to extend Hunter's calculations and related ones by Lecar and Franklin¹³, to study the capture and ejection of satellites by Saturn and to try to determine the efficiency of transfer of asteroids outwards. In a crude attempt to estimate the efficiency, I shall use Hunter's hypothesis¹⁰ that the distribution in mean motion of the asteroids was originally roughly Gaussian and that the present dearth of asteroids

with mean motions between 550 and 300 arc s d⁻¹ is due to capture by Jupiter and subsequent ejection to an outer belt. (The small group at about 450 arc s d⁻¹ is at the 3/2 commensurability and is supposed to be less easily perturbed.) From Hunter's Fig. 1 (ref. 10) I adopt

$$N(n) = 9 \exp(-[(n-640)/125]^2)$$

where n is the mean motion in arc s d⁻¹ and $N(n)$ is the number of asteroids in unit range of n .

The number originally in the range 550 to 300 arc s d⁻¹ would have been about 330, hence, there being now about 50 in that range, about 280 would seem to have been transported outwards, that is, nearly 15% of an original total of nearly 2,000. If transport by Saturn is equally efficient a further 15%, or about 40 asteroids, would be expected to have been transported to a belt between Saturn and Uranus. Of course, some asteroids may be permanently captured in this process. The four outermost satellites of Jupiter, and Phoebe, the outermost satellite of Saturn, may be such captured asteroids¹⁰ (but see ref. 14). If so, their relative numbers (1:4 or 25%) should reflect the relative numbers of asteroids available for capture in the belt inside them (15% if the above picture is correct). Given the small numbers and the very rough nature of the estimate, this agreement is satisfactory.

If the above picture is correct, then Chiron should be just one of a small but significant group of perhaps 40 asteroids with orbital semi-major axes larger than that of Saturn. Other members of the group would probably be fainter than Chiron and correspondingly harder to detect. However, there should also be a larger population of nearly 300 brighter asteroids, with apparent magnitudes up to about 15 and orbits between Jupiter and Saturn, perhaps concentrated into two belts¹³. These asteroids would have larger mean motions than Chiron and so be rather easier to detect. It would be worthwhile to make a systematic search for these, although the number estimates are extremely rough.

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Determination of water adsorption sites in wood by a hydrogen–deuterium exchange

It is important in the field of forest products science to know the sorption mechanism of water in wood. Adsorption of water onto hygroscopic natural polymers, such as wood and cellulose, is thought to depend entirely on the hydroxyl groups on the surface¹. The mechanism of water adsorption in wood can therefore be studied by evaluating the quantity and the state of the adsorption sites, hydroxyl groups. Until now, in order to measure the number of adsorption sites of water in wood, it has been usual to draw a sorption isotherm from which the adsorbed area can be

calculated assuming monomolecular layer adsorption according to the BET theory². Little is known of the sorption mechanism on the molecular level, however. We report here the use of a hydrogen–deuterium exchange method to determine the adsorption sites of water in wood. Hydrogen–deuterium exchange is a well established method which has been widely used to determine hydroxyl group accessibility^{3,8,9}.

The infrared spectra of wood show that all potentially sorbible hydroxyl groups are mutually bonded in oven-dry wood⁴. In the presence of water vapour these bonds are broken by the intrusion of the H₂O molecule. By using D₂O instead of H₂O and raising the relative vapour pressure in D₂O to the desired level and then decreasing it, information on the number of sites involved can be obtained. The significance of decreasing the relative vapour pressure in D₂O is important because only by doing this can the number of locations of hydroxyl linkage in the cellulose molecule to H₂O in the cell wall be estimated through the increase in molecular weight induced by substitution of D₂O for H₂O. Only hydroxyl groups in the cellulose molecule at sites where the cellulose bond is broken will react with D₂O.

The amount of deuterium-exchangeable hydroxyl groups per g of wood was estimated in given relative vapour pressure. To determine the number of deuterated hydroxyl groups, the change in weight of the deuterated wood sample was measured gravimetrically using a quartz spiral spring balance with an evacuation device, in which the environment can be changed so as to produce a desired relative D₂O vapour pressure. Any relative vapour pressure of D₂O is produced by controlling the temperature of D₂O reservoir connected to the sorption chamber. The gain in weight of the deuterated sample from vacuum dry state was considered to be related to the number of adsorption sites present.

Figure 1 shows the extent of deuterium exchange for the conditioned Japanese cypress (*Chamaecyparis obtusa* Endl.) under the various relative D₂O vapour pressures. It can be seen that the amount of deuterium exchange for the wood increases with increasing relative D₂O vapour pressure up to about 0.6, and then reaches an equilibrium point at approximately 0.6, indicating that the exchange is completed.

Our results suggest that new adsorption sites are continuously created with increasing relative vapour pressure up to about 0.6 where D₂O exchange is complete, and that no new sites are created at a D₂O relative vapour pressure above about 0.6. (Assuming that the amount of deuterium exchange is proportional to the number of adsorption sites.)

A similar result is obtained for H₂O and D₂O adsorption when the sorption isotherm of H₂O and D₂O for wood and for poly *N*-*t*-butylacrylamide⁵ are expressed on mole basis. As the bond energies of a hydrogen bond and a deuterium bond are similar⁶, it is probable that new adsorption sites for D₂O are formed in a similar manner to those for H₂O adsorption.

At the equilibrium point of the deuterium exchange (Fig. 1), monomolecular H₂O adsorption is thought to take place. Assuming that one water molecule adsorbs one adsorption site, the

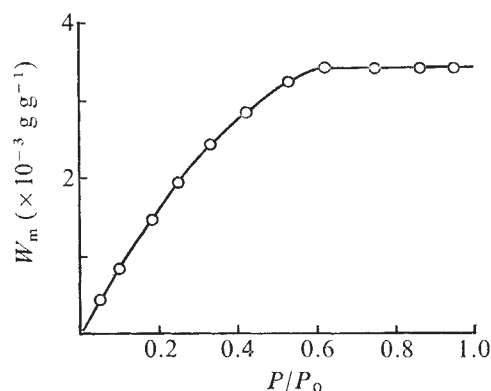


Fig. 1 Deuterium exchange (W_m) for Japanese cypress plotted against relative D₂O vapour pressure (P/P_0).

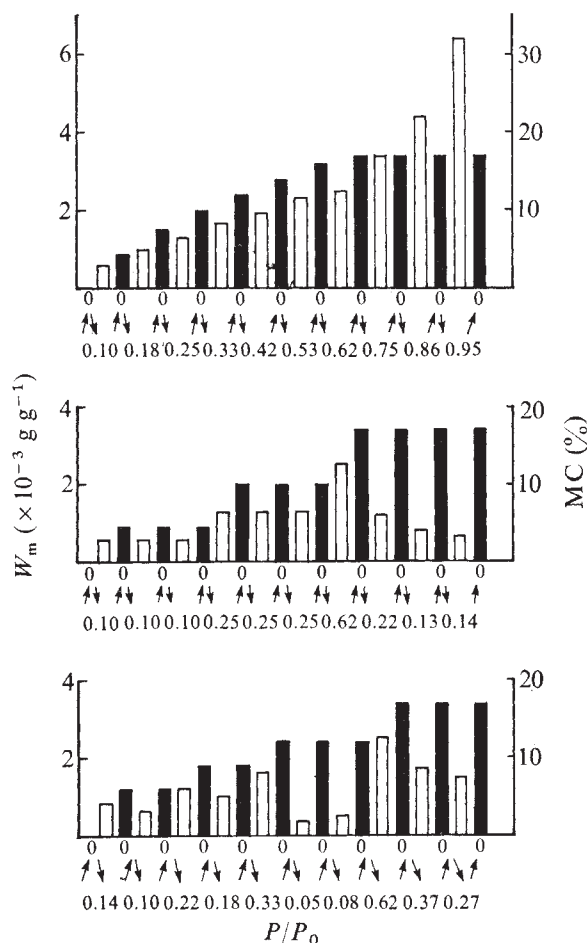


Fig. 2 Deuterium exchange (W_m) for Japanese cypress when treatment was repeated at an arbitrarily chosen relative D_2O vapour pressure (P/P_0). Open columns, moisture content (MC) at an arbitrarily chosen relative D_2O vapour pressure. Solid bars, amount of deuterium exchange (W_m).

surface area (S) on H_2O adsorption can be calculated if the cross sectional area of a water molecule is taken as $10.53 \times 10^{-2} \text{ nm}^2$ (ref. 7); as the monomolecular layer (W_L) for H_2O adsorption is $6.28 \times 10^{-2} \text{ g g}^{-1}$ (from Fig. 1), the surface area for H_2O adsorption becomes $221 \text{ m}^2 \text{ g}^{-1}$.

On the other hand, if one applies BET method to the H_2O adsorption isotherm the W_L value and calculated S become $4.97 \times 10^2 \text{ g g}^{-1}$ and $175 \text{ m}^2 \text{ g}^{-1}$, respectively. These values agree well with those obtained from the hydrogen-deuterium exchange method considering that the monomolecular layer calculated by BET method does not necessarily correspond to the endpoint of formation of the new adsorption sites. Of the methods so far used, only the hydrogen-deuterium exchange method gives the change and the equilibrium point of formation of the adsorption sites.

Figure 2 shows the amount of deuterium exchange when adsorption was repeated at the arbitrarily determined relative D_2O vapour pressure. The following conclusions may be drawn from these results. (1) If the wood sample was first deuterated at some relative D_2O vapour pressure below about 0.6, and the relative D_2O vapour pressure was then lowered, the amount of deuterium exchange remained in the first state. (2) If the wood sample was repeatedly deuterated at the same relative D_2O vapour pressure below about 0.6, the amount of deuterium exchange remained constant. (3) If the wood sample was deuterated at a higher relative D_2O vapour pressure than the previous treatment, but lower than 0.6, the amount of deuterium exchange increased.

It is clear from these results that at a relative H_2O vapour pressure below about 0.6, new H_2O adsorption sites are formed in a fixed order, and the formation of these adsorption sites is

verified by the uniform value corresponding to a certain relative H_2O vapour pressure. The new adsorption sites are not formed at any relative H_2O vapour pressure above about 0.6.

This hydrogen-deuterium exchange method can be easily applied to the materials which contain deuterium exchangeable functional groups, OH or NH groups, so the method provides information on the monomolecular layer of adsorption as well as on the formation of adsorption sites on H_2O adsorption.

This method of labelling adsorption sites is thus extremely effective for the elucidation of the sorption mechanism of the hygroscopic natural polymers.

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Analysis of a possible Sun-weather correlation

XANTHAKIS¹ has reported a striking, if complex, relationship between solar activity and Northern Hemisphere precipitation. This resulted in a strong negative correlation between rainfall and solar activity found in the latitude belt of 60° to 70°N . Other belts showed pronounced positive correlations, or a change from negative to positive correlation. We decided that these correlations needed a totally independent confirmation², and so we repeated the analysis, using a more extensive data base. Xanthakis has since published a more extensive survey of global precipitation and solar activity³. This also contains detailed information on his methods of analysis, and extends the study to the Southern Hemisphere, where further verification of the association is claimed. Our studies reported here also encompassed both hemispheres and our results show only a general similarity with those of Xanthakis.

Our studies were based on annual precipitation records from 2,548 stations around the world for the period 1850-1973. We used the archived magnetic tape records available at the National Center for Atmospheric Research (NCAR) in Boulder, Colorado. We analysed all available data in each 10° latitude belt from pole to pole. However, we felt that data coverage was only adequate for reliable interpretation from about 20°S to 70°N over the full time span.

For each 10° latitude band we constructed a time series X_k ($k = 1, 124$), representing, for each year, the average amount by which total annual precipitation at each of the stations exceeded the station's 124 yr minimum. In other notation, for each latitude belt:

$$X_k = \frac{1}{N} \sum_{j=1}^N (R_{kj} - R_j) \quad (1)$$

where N is the number of stations reporting during a given year; j ,

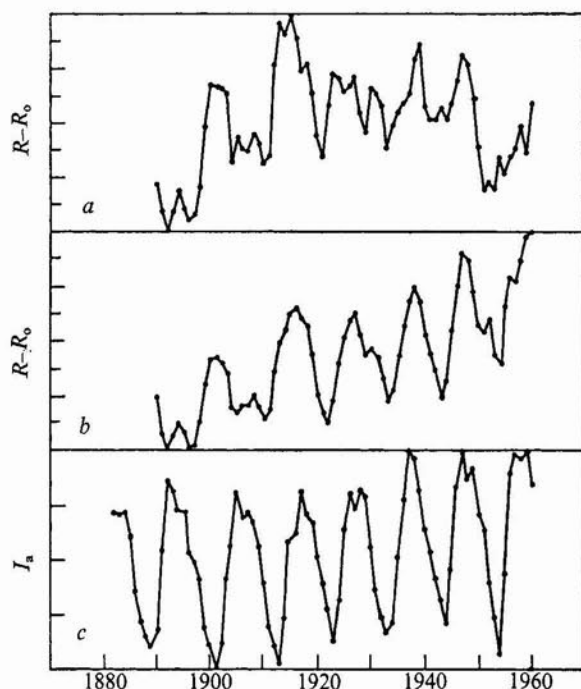


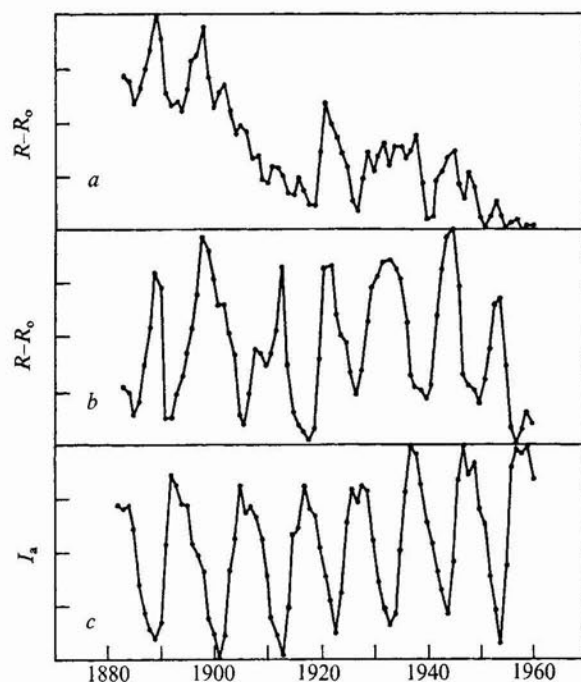
Fig. 1a. Precipitation time series for the latitude band 50°N to 60°N as computed by the authors. b. Precipitation time series for the same latitude band as computed by Xanthakis. c. The solar activity parameter of Xanthakis.

the station index; k , the year; R_{kj} , the precipitation for the year and station; R_j , the lowest annual precipitation reported for the j th station over the entire 124 yr period.

We used X_k rather than the simple average, because X_k was used by Xanthakis. As X_k is merely the average total annual precipitation minus the average 124 yr minimum, this parameter offers no advantage whatever over the raw average of total annual precipitation.

Figure 1a shows the resulting time series for the latitude belt 50–60°N. For comparison, Fig. 1b shows the corresponding curve

Fig. 2a. Precipitation time series for the latitude band 60°N to 70°N as computed by the authors. b. Precipitation time series for the same latitude band as computed by Xanthakis. c. The solar activity parameter of Xanthakis.



from Xanthakis, and Fig. 1c, the solar activity parameter of Xanthakis. Figure 2 gives the corresponding data for the latitude belt 60–70°N.

The disparities between our precipitation data in Figs 1 and 2 and those of Xanthakis are substantial. Although there is some similarity, it is doubtful whether one would draw the same conclusions from our precipitation record: namely that in the band 50–60°N, solar activity is negatively correlated with precipitation between 1880 and 1913 and positively correlated between 1914 and 1960 and, that in the band 60–70°N solar activity and precipitation are negatively correlated. There is a hint of this in our analysis, but it is weak.

As a further step, we carried out a spectral analysis according to the techniques of Blackman and Tukey⁴ on the time series for each of our latitude bands. The maximum lag was 31 yr. Significance levels for the power spectral estimates of a particular series were computed using either a white or red-noise criterion, whichever was appropriate. Figure 3 shows the number and frequency distribution of power spectral estimates significant at the 95% level. The most notable feature of the power spectrum analysis is the complete absence of significant spectral peaks with periods between about 6 and 20 yr, casting more doubt upon the existence of a solar cycle variation in global precipitation.

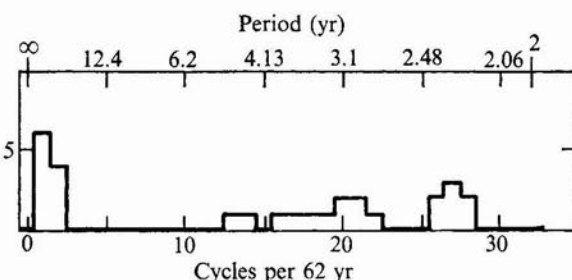


Fig. 3 Number of power spectral estimates significant at the 95% level, as a function of period, for all 14 time series represented in our analysis.

From these comparisons, we are forced to be sceptical about the claims of correlation of Xanthakis. We hope that the search for Sun-weather mechanisms will continue, however, because there is strong empirical evidence in recent papers by Wilcox⁵, Hines⁶ and others for significant connections that need theoretical understanding. We are convinced that the western USA exhibits a significant 20–22 yr drought periodicity that may be associated with the so-called 'double sunspot cycle' of the same periodicity. A recent study by Mitchell (unpublished) indicated that this relationship is on firm statistical ground.

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An investigation of the astronomical theory of the ice ages using a simple climate-ice sheet model

THE astronomical (or Milankovitch) theory of the Quaternary ice ages has been the subject of several recent studies using simple climate models¹⁻³; this approach is potentially more complete than earlier 'insolation curve' investigations⁴. The external forcing of this theory, that is, variations in incident sunlight due to secular earth orbit perturbations, is well known⁵, and deep-sea sediment core records^{6,7} provide a good data base for testing the model results. If any component of this data can be linked conclusively to the astronomical forcing, this would provide useful knowledge of the sensitivity/stability of the present climate² (for example, to several types of human perturbations). This letter describes an attempt to incorporate the ice sheets explicitly into a simple climate model's description of the global weather, guided by the fact that ice sheet variations are the principal features of the ice ages, but with these variations forced mainly by relatively small changes in the weather. The results combine features of previous results of purely climatological^{1,2} and purely glaciological models^{3,8}, with the ice sheets' albedo feedback, topography and dynamics all being important in the present model. The curve of the model's ice ages is similar to previous model curves^{2,3}, comparing favourably with some aspects of the deep-sea record but not simulating the dominant ~100,000 yr 'sawtooth' cycle of this data at all.

The weather of 1 yr is described by a zonally symmetric, time-dependent, one-level energy-balance equation which is

solved numerically for sea-level air temperature T_s as a function of latitude, l , and month. Details of the model are listed under Fig. 1. Net infrared cooling and atmospheric/oceanic meridional heat transport are parameterised simply in terms of T_s and $\delta T_s/\delta l$. Up to this point, the model is essentially a time-dependent version of North's⁹ annual average model. Seasonally varying snow cover in land (which affects planetary albedo) and on the ice sheets is included diagnostically by parameterising monthly snowfall and snowmelt in simple ways. For simplicity, the model's meteorological parameters represent two Northern Hemispheres patched together, as primarily Northern Hemispheric glaciations are under investigation. The meteorological parameters were adjusted to fit the present climate of the northern hemisphere, using zonally averaged seasonal data^{10,11} where available. The weather of 1 yr, then, is a unique function of (1) the latitudinal and seasonal distribution of incoming sunlight and (2) the current latitudinal extent of the ice sheets (through planetary albedo).

The ice sheets are treated following Weertman^{3,12}. Real ice sheets grow or decay in response to the forcing of net annual accumulation and ablation, and flow outwards under their own weight, on time scales of thousands to tens of thousands of years. The model ice sheets' flow is approximated as perfect plasticity¹², which constrains their height profiles to be parabolic

$$h(x) = [\lambda(L - |x|)]^2 \quad (1)$$

where λ is a constant depending on the yield stress of ice, L is the half-width of the ice sheet, and x is the latitudinal distance from its centre. The model's Antarctic ice sheet is centred on the South Pole and allowed to extend out only to 70°S. A

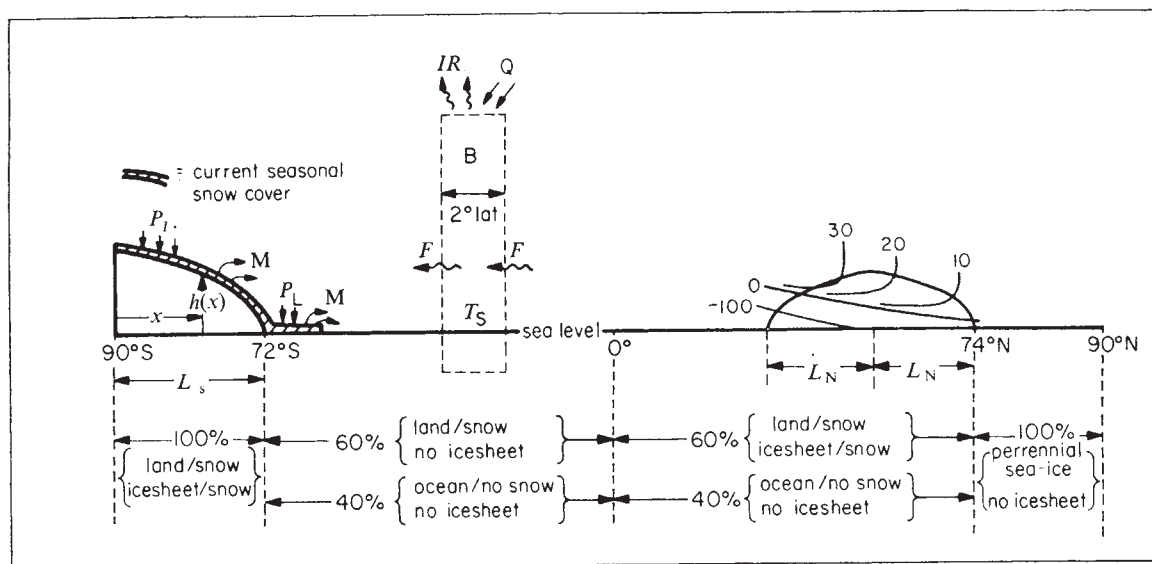


Fig. 1 Schematic diagram (latitude against height) showing processes and surface boundary conditions of the climate model. (Although drawn flat, the model computations are for a spherical globe). Surface boundary conditions are shown; these affect the planetary albedo feedbacks of ice sheets and seasonal snowcover, and, except near the poles, represent the mean zonally averaged land-ocean ratio of the real Earth from ~45°N to ~65°N. The model neglects seasonal variations of sea-ice and palaeo-variations of cloudiness. B: All quantities below are computed for zonal rings, B, 2° latitude wide, extending up out of the atmosphere and down to the zonal mean of the seasonal oceanic thermocline and thermal skin-depth of land. However, the prognostic energy-balance equation for T_s (zonal mean sea-level air temperature in °C) is solved spectrally, only keeping the first three spatial eigenfunctions (legendre polynomials $P_0(s)$, $P_1(s)$, $P_2(s)$, where $s = \sin(\text{latitude})$). Any finer spatial resolution would be unrealistic⁹ because of the crude parameterisations below. A constant heat capacity equivalent to a liquid water depth of 11 m is used for all latitudes. Q, Monthly mean sunlight incident at the top of the atmosphere. Planetary albedo is 0.62 for snow or ice sheet surfaces, and $0.31 + 0.08 P_2(s)$ for land and ocean surfaces⁹. P_L , Snowfall rate on land, equal to the present observed zonally averaged mean annual precipitation rate if $T_s \leq 0^\circ\text{C}$; equals zero if $T_s > 0^\circ\text{C}$. P_I , Snowfall rate on ice sheet surfaces; $P_I - P_L$ for model runs in Fig. 2. M, Monthly mean ablation ('snowmelt') = $\max(0, a Q + b T_s^* + c)$. a, b, c are constants based on a crude surface energy balance¹⁵ and actual glacial measurements¹³⁻¹⁷. $a = 0.32 \text{ (g cm}^{-2} \text{ month}^{-1}) \text{ (Wm}^{-2})^{-1}$, $b = 10.0 \text{ (g cm}^{-2} \text{ month}^{-1}) \text{ (}^\circ\text{C)}^{-1}$, $c = -47.0 \text{ g cm}^{-2} \text{ month}^{-1}$. The value of a allows for multiple reflections off some standard cloud deck¹⁸, and is for a melting ice surface albedo of ~0.65. T_s^* equals T_s corrected for ice sheet height using a constant lapse rate of $-6.5^\circ\text{C km}^{-1}$. L_s , L_N : Half-widths of the ice sheets, as in equation (1). The southern sheet is circularly symmetric about the South Pole, and the northern sheet is two-dimensional (as in Weertman⁹). North Cap: typical pattern of net accumulation minus ablation is sketched; units are $\text{g cm}^{-2} \text{ month}^{-1}$. High ice sheet elevations protect the central regions from ablation; precipitation rates P_I decrease towards the pole.

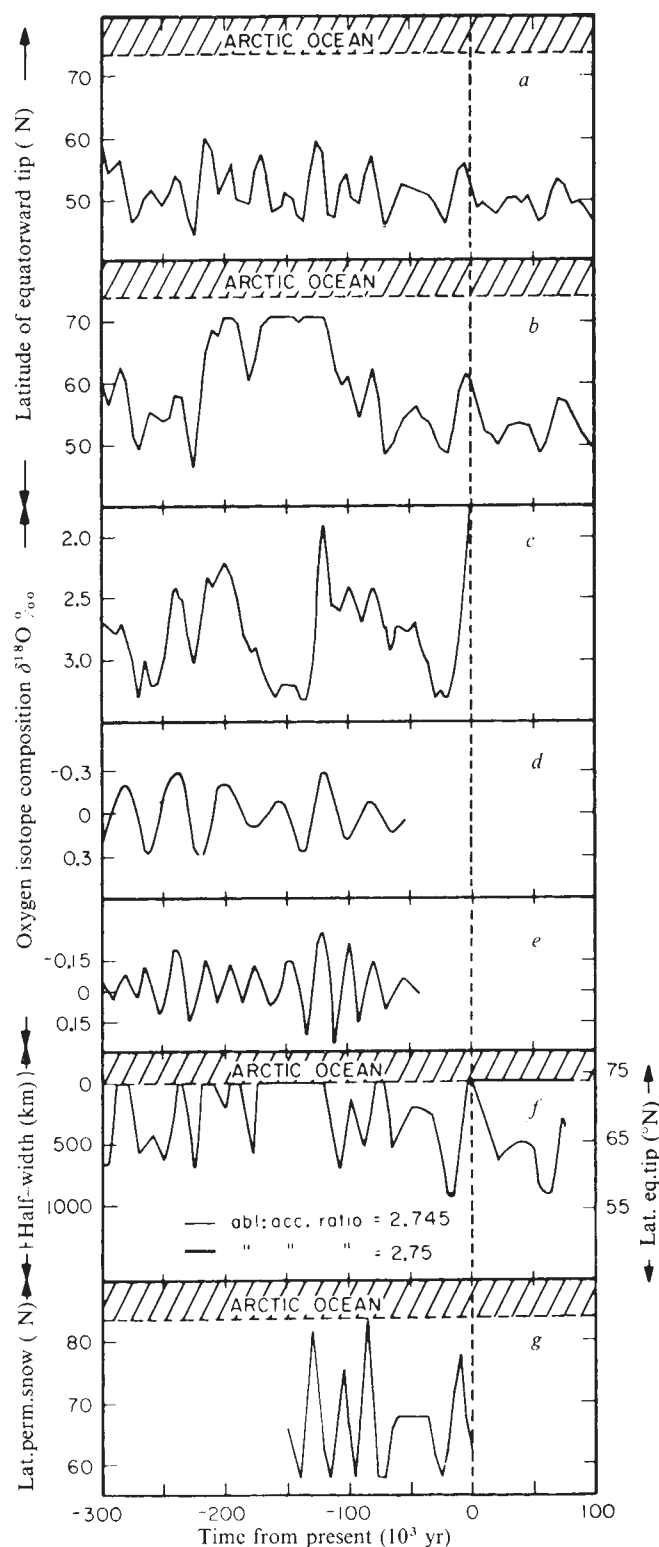


Fig. 2a, b, Model runs for the last 300,000 yr using actual Earth orbit perturbations, showing the fluctuating latitude of the equatorward tip of the Northern Hemisphere ice sheet. (The runs were extended 100,000 yr into the future, but ice cap response in this period will probably be modified by human activities if present-type civilisations survive). Curve (a) is for a value of the ice sheet profile parameter $\lambda = 4.9$ m, and curve (b) for $\lambda = 4.2$ m. (These runs took approximately 4 min of computer time per 100,000 yr on an IBM 370). Curves (c), (d) and (e) are the palaeo-record of $\delta^{18}\text{O}$ (oxygen isotope ratio) of planktonic foraminifera in a deep-sea core, and its $\sim 40,000$ yr and $\sim 23,000$ yr frequency components (from ref. 7). f, Two runs of Weertman's³ glaciological model; g, Suarez and Held's² model 'palaeoclimatic record', that is, fluctuations of their seasonal minimum (summer) extent of snowcover on land. Curves (c) to (g) have been redrawn from the original papers.

typical Northern Hemispheric continental glaciation is modelled by constraining a model ice sheet to extend equatorward from 75°N (corresponding to the Arctic Ocean shoreline).

The model is run by working out one year's weather once every 2,500 yr. On the ice sheets, accumulation is set equal to the present observed mean precipitation at each latitude, and monthly ablation is computed using the same parameterisation as for snowmelt on land, but with T_s corrected for ice sheet height using a constant lapse rate of $-6.5^\circ\text{C km}^{-1}$ (see Fig. 1 legend). The total accumulation minus ablation integrated over an ice sheet's surface area determines volumetrically how much the sheet advances or retreats in the 2,500-yr time-step³, assuming the ice sheets always maintain plastic profiles (1) with varying L . (Neither of Weertman's³ two 'stagnant ice sheet' possibilities ever occurs in the present model.)

Two typical model runs using actual orbital perturbations⁵ of the last 300,000 yr are compared in Fig. 2 with a deep-sea core record that reflects global ice sheet volume changes⁷. Fourier spectra of this and other core data show a dominant peak at a period of $\sim 100,000$ yr, with smaller peaks at $\sim 40,000$ yr and $\sim 23,000$ yr (ref. 7). As shown in Fig. 2, many maxima and minima of the $\sim 40,000$ yr and $23,000$ yr frequency components of the core record coincide with maxima and minima of both the present model's curves and also those of Weertman's³ and Suarez and Held's². These simple models, then, seem to simulate correctly the phase (and approximate amplitude) of the higher frequency components of the core data, with the $\sim 40,000$ yr and $\sim 23,000$ yr responses tied to variations in obliquity and precession, respectively. However, the dominant $100,000$ yr 'sawtooth' cycle of the data^{6,7} is not simulated by these models at all.

Temperature differences in T_s between maximum and minimum ice sheet extents that occur in the present model's runs are $\sim 9^\circ\text{C}$ in the polar region's summer, $\sim 6^\circ\text{C}$ in mid-latitude summer, and $\sim 1^\circ\text{C}$ in the tropics. These compare favourably with equivalent Climap¹³ data, (remembering that T_s is a smeared land-ocean sea-level air temperature).

Figure 2 also shows two ice-age curves of Weertman's glaciological model³. In both his and the present model, the amplitude of the ice sheet response to orbital perturbations is limited by the volume-inertia of ice required to change the ice sheet's size. To achieve this large a response, Weertman had to assume unrealistically large (by $\sim 100\%$) accumulation rates; equivalently, for the present model, isostatic depression of the land underneath the ice sheet was ignored, and unrealistically small values of λ were used ($\lambda \approx 5$ m corresponding to an ice yield stress of ~ 0.2 bar). Elevation profiles of Antarctica and Greenland today imply a value of $\lambda \sim 10$ m, which, when used in other model runs (not shown) reduced the amplitude of the ice sheet response to $\sim 7^\circ$ in latitude). Parts of Weertman's curves are very sensitive to changes (0.2%) in his ablation: accumulation ratio; in a similar way, parts of the present model's curves are sensitive to changes ($\sim 7\%$) in the value of λ (see Fig. 2), which affect ablation through the lapse rate correction to T_s for ice sheet height.

Figure 3 shows how the northern ice sheet would grow or decay as a function of its size, for various combinations (mostly extreme) of the orbital elements. If the orbital elements were to remain fixed at one of these combinations, the ice sheet size would move along the corresponding curve until it either arrives at a stable point 'S' or recedes to insignificance 'I'. At points 'U', the ice sheet is in unstable equilibrium. This feature, of two equilibrium ice sheet sizes (for a fixed orbital element combination) with the smaller sheet being unstable and the larger one stable, is analysed by Weertman³ as a consequence of ice sheet topography, but the effect may be slightly enhanced by albedo feedback⁹. As Suarez and Held's² model neglects time lags due to ice sheet volume changes, their ice age curve would be analogous to the curve generated by the intersections 'S' of successive Fig. 3-type curves (as the actual orbital elements varied) with the 'zero-regime' axis.

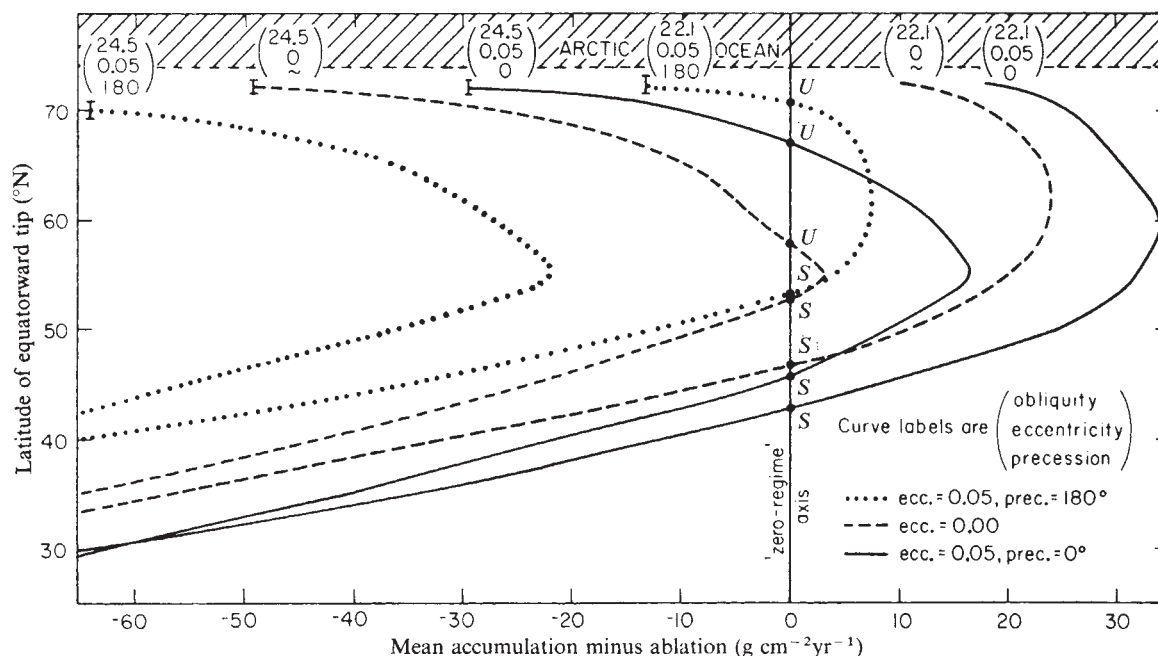


Fig. 3 Stability diagram showing northern hemispheric ice sheet 'regime' as a function of its latitudinal extent, for various combinations of orbital obliquity, eccentricity and precession. (The 'regime' is the net annual accumulation minus ablation averaged over the ice sheet surface area. Precession is defined here so that it is zero when the northern hemispheric winter solstice coincides with perihelion, and 180° when it coincides with aphelion.) For Fig. 3, the model Antarctic size is fixed, extending out to 70°S. In all models runs (for example, Fig. 2), the Antarctic's regime was positive, so the ice sheet always remained at this maximum size.

Work to explore the sensitivity of the model to other types of parameterisations is in progress. Other ways of parameterising precipitation have been tried, using simple functions of T , and $\delta T/\delta t$ (and even h and $\delta h/\delta t$) that crudely represent atmospheric stirring of water vapour at constant relative humidity. These give essentially the same ice-age curves as the empirical precipitation version above, which implies that ablation is the most important mechanism in the astronomical forcing. The model's sensitivity to changes in the solar constant is similar to North's⁹ and Suarez and Held's², with the Northern Hemispheric ice sheet growing to the equator if the solar constant is reduced by ~5% (with the present orbital elements). The most serious discrepancies of the model's climate from reality stem from having no land-sea heat capacity contrast (for example, for the present Northern Hemisphere, the model's maximum temperatures T , occur at the end of August, whereas the central regions of continents are hottest in mid-July. So, on the model's ice sheet surfaces, months of maximum temperature lag too much behind the months of maximum sunlight, which reduces the ice sheet ablation). That, and a more general description of ice sheet profiles using some standard ice-flow equation¹⁴, seem like important refinements of the model.

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Consumption of dissolved nitrous oxide in an anoxic basin, Saanich Inlet, British Columbia

DATA on dissolved nitrous oxide (N_2O) in the ocean indicate that its distribution is strongly affected by oxygen-controlled biochemical reactions¹⁻⁴. N_2O has been measured in oxygenated, open ocean waters of the Atlantic and Pacific Oceans¹⁻³, and in the oxygen deficient waters of the eastern tropical North Pacific⁴ but no measurements of N_2O in anoxic marine environments have yet been made. This report presents measurements of N_2O from Saanich Inlet, an intermittently anoxic basin on the southeastern side of Vancouver Island, British Columbia, and evidence for its consumption during denitrification.

Anoxic conditions develop in basins where circulatory replenishment of the deep water is restricted and the oxidation of organic materials is rapid relative to the rate of supply of dissolved oxygen. Such basins are characterised by an upper oxygenated layer in which oxygen concentrations rapidly diminish with depth; an interface layer, containing very little or no oxygen, where denitrification might take place; and a bottom anoxic layer, containing no oxygen, nitrate or nitrite, in which sulphate reduction might proceed^{5,6}. In Saanich Inlet, a shallow sill (~70m) at its northern end restricts water exchange with the adjacent Satellite channel causing stagnation of the inlet's deep water in the spring and summer. The inlet is flushed with oxygenated water in late summer-early autumn⁷. The present study was undertaken in July 1977, (Cruise TT-119 of the University of Washington's RV Thompson) when anaerobic conditions, as shown by H_2S and ammonia accumulation (Fig. 1), prevailed in the deep water of the inlet. The depth

of the interface layer was found from continuous measurements of oxygen and nutrients in water sampled by the University of Washington continuous pumping system. Subsequently, discrete bottle samples were taken for measurements of N_2O , oxygen, H_2S , nutrients, temperature, and salinity; near the interface, the sampling interval was 5 m.

The interface layer at about 135–140 m at the two stations investigated can be identified from discontinuities in all the chemical variables measured except phosphate (Fig. 1). Nitrate concentrations drop to zero at the interface and a small secondary nitrite maximum ($\sim 0.1 \mu\text{mol l}^{-1}$) at station 2 indicates denitrification^{5,6}. From the vertical extent of this secondary nitrite maximum and the samples which contained both oxygen and H_2S it appears that the thickness of the interface layer is no more than 10 m. At station 1, a secondary nitrite maximum was not found from the bottle samples but its presence ($\sim 0.04 \mu\text{mol l}^{-1}$) at the interface was established from the continuous measurements.

The vertical N_2O distributions are similar at the two stations (Fig. 1). N_2O concentrations gradually increase from the surface to just above the interface, reaching 17.8 nmol l^{-1} and 20.4 nmol l^{-1} at stations 1 and 2, respectively (Table 1). Between the surface and the N_2O maxima, N_2O concentrations are inversely related to dissolved oxygen concentrations, similar to what has been found in other areas of the ocean¹⁻⁴. Across the interface layer, the N_2O concentrations decrease drastically, reaching zero in the deep water. In terms of per cent N_2O saturation, this corresponds to a drop from about 200% saturation to zero (Table 1). This is the first time that zero concentrations of N_2O have been reported in the marine environment at this detection limit⁸. The lowest N_2O saturation levels ever recorded in the open ocean, about 50%, were from the core of the oxygen minimum of the eastern tropical North Pacific⁴.

Slightly above and within the interface layer, the N_2O , oxygen, H_2S , and nutrient relationships displayed in Fig. 1 clearly indicate biological consumption of N_2O . At the two stations, the sharp decrease in both the nitrate and N_2O concentrations begins in the 10 m interval above the interface.

Table 1 N_2O concentrations and per cent N_2O saturation at two stations in Saanich Inlet

Station 1			Station 2		
Depth (m)	N_2O (nmol l ⁻¹)	N_2O saturation (%)	Depth (m)	N_2O (nmol l ⁻¹)	N_2O saturation (%)
Oxygenated					
1	11.3	140			
6	12.0	140			
16	13.7	155	0	11.6	142
26	14.0	154	10	13.2	155
46	14.7	158	30	14.1	153
71	16.4	168	50	14.2	149
96	17.4	177	100	19.3	196
106	17.8	181	110	20.4	208
111	17.4	177	115	18.3	186
116	17.2	175	120	18.9	192
121	16.5	168	125	17.0	174
126	17.3	177	130	10.2	104
131	13.9	142	135	2.9	30
Anoxic					
136	0.3	3	140	2.0	20
146	0.1	1	146	1.0	10
171	0.1	1	150	0.0	0
196	0.0	0	169	0.0	0
			179	0.0	0

Station locations: $48^\circ 35.1'N$, $123^\circ 30.1'W$ (1); $48^\circ 33.1'N$, $123^\circ 32.7'W$ (2). Per cent saturations are relative to a marine air mixing ratio of 278 p.p.b.v. measured in Saanich Inlet using the same analytical procedure used for seawater analysis⁸. The N_2O solubility coefficients in seawater were calculated by fitting Weiss' equation (ref. 20) to the data of Markham and Kobe²¹ for the solubility of N_2O in aqueous sodium chloride solutions.

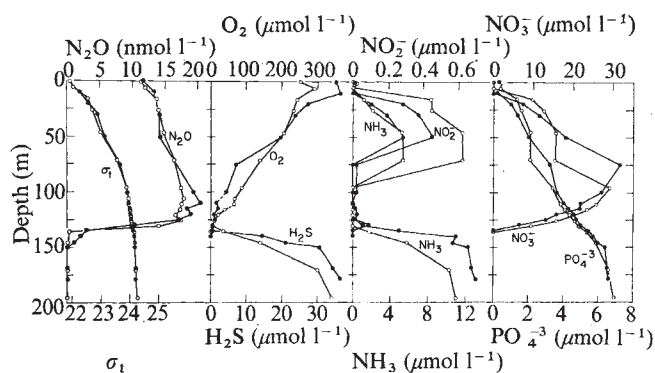


Fig. 1 Vertical distributions of N_2O , oxygen, H_2S , nutrients and density at $\circ$, Station 1; $\bullet$, Station 2 in Saanich Inlet in July 1977. N_2O was measured by electron capture gas chromatography⁸. The method's precision is about 2% (1 sd), its detection limit is about 0.05 nmol l^{-1} ; and its accuracy is estimated at $\sim 3\%$. Nutrients were measured with the University of Washington AutoAnalyzer system. Temperatures are from CTD casts. Dissolved oxygen was measured both by the Winkler procedure and by the colorimetric procedure of Broenkow and Cline⁹. The latter method is suitable for very low oxygen levels where the Winkler method gives systematically higher values. The broken line in the oxygen curves indicates the concentration range above which the Winkler procedure was used and below which the colorimetric procedure was used. $\sigma_t = (\rho - 1) \times 10^3$, where ρ is the density of the water.

In this depth range, oxygen concentrations are lower than $10 \mu\text{mol l}^{-1}$, a range favourable for denitrification^{10,11}, but H_2S is absent. The presence of denitrifying bacteria at this depth range was confirmed by R. J. Ozretich (personal communication) in experiments carried out at sea a few hours after the N_2O measurements were made. One may therefore conclude that N_2O is consumed during denitrification.

Barbaree and Payne¹², and Payne *et al.*¹³ demonstrated that N_2O can serve as the terminal electron acceptor for the anaerobic growth of marine denitrifiers. Cohen and Gordon's⁴ study in the eastern-tropical North Pacific indicated biological consumption of N_2O around the core of the oxygen minimum where denitrification is the predominant respiratory mechanism^{11,14,15}. This was demonstrated by the association of minima in the N_2O profiles with near zero oxygen concentrations and secondary nitrite maxima. The present findings represent an extreme case where denitrification completely removes the nitrate, nitrite and dissolved N_2O in the water. The presence of low levels of N_2O in the upper part of the sulphide-bearing waters is most probably the result of diffusion across the interface.

Much effort has recently been devoted to the identification of the natural sources and sinks for atmospheric N_2O because of its involvement in the chemistry of stratospheric ozone. Publications concerned with the potential weakening of the ozone layer following the introduction of anthropogenic N_2O into the atmosphere have emphasised that a major natural sink for atmospheric N_2O has not been identified¹⁶⁻¹⁹. So far all N_2O measurements in surface waters of the ocean have revealed varying degrees of supersaturation and have failed to disclose an area where the sea surface acts as a sink for atmospheric N_2O ¹⁻⁴. In Saanich Inlet, N_2O is highly supersaturated in the oxygenated layer, indicating that it is being produced in that layer, and apparently the inlet is a local source for N_2O in the atmosphere. The disappearance of dissolved N_2O in the anoxic layer raises the possibility that local sinks for atmospheric N_2O might exist in shallow aquatic environments, such as salt marshes, where denitrification and anoxic conditions might occur close to the air-water interface.

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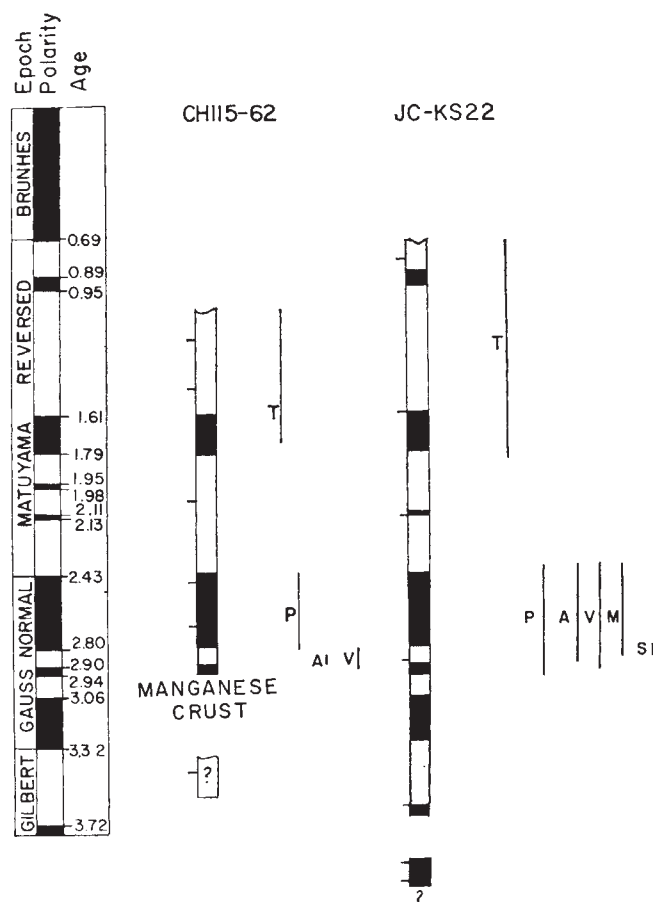
Late Pliocene climate and south-west Atlantic abyssal circulation

THE late Pliocene is characterised by a global climatic cooling¹⁻⁴ and by widespread erosion of abyssal sediments by Antarctic Bottom Water (AABW)⁵⁻⁶. The global cooling is dated between 2.5 and 3.0 Myr based on the age of the oldest tillite in Iceland⁷, the initiation of glaciation in the Sierra Nevada of the USA⁸, faunal changes in Antarctic and sub-Antarctic deep-sea cores⁹⁻¹¹, and a change in carbonate preservation in the equatorial Pacific¹². This cooling may be synchronous with the development of the Northern Hemisphere ice sheet which has been dated by the first appearance of ice-rafted debris in the North Atlantic at 3 Myr followed by a major influx at 2.6 Myr (ref. 13), and by a large change in the oxygen isotope record of benthic foraminifera indicating an increase in ice volume at ~ 2.6 Myr (refs 2, 3). The global cooling and deep-sea erosion events have been identified in widely separated studies and the ages assigned to each are based on biostratigraphic zonations with long durations. The apparent synchronicity of the two events (within the limits of the methods used) has led to the suggestion of a cause and effect relationship with the global cooling driving the increase in abyssal circulation^{14, 15}. In an attempt to find the cause of the relative timing of both the climatic and circulation events, which has not been determined with sufficient stratigraphic resolution, we have examined two late Pliocene sections in the south-west Atlantic.

The Late Pliocene sections of two piston cores from the flank of the Vema Channel in the south-west Atlantic (Table 1) were examined within a detailed stratigraphic framework (Fig. 1). The results show that the 8-cm thick lithified layer capped with a 2-mm manganese crust recovered in core Chain 115-62 can be dated 2.90-2.94 Myr. This manganese crust is apparently synchronous with a major lithologic boundary between carbonate-free and high carbonate sediment in core Jean Charcot GB-KS22 (Fig. 2). Therefore, a deepening of the calcite compensation depth (CCD) accompanied the resumption of sedimentation above the manganese crust.

The presence of the manganese crust suggests a hiatus in sedimentation caused by either the absence of carbonate sedimentation below the CCD as observed in the red clay facies of the North Pacific⁶, or erosion by bottom currents as shown in the Southern Ocean²⁰. In principle, the particle size and the degree of alignment of the long axis of magnetic particles may be used to distinguish a bottom current origin, as an increase in velocity will result in coarser sediment and a more efficient alignment of long magnetic grains¹⁸. The coarsest particle sizes and the highest alignment in core CH115-62 were found above the manganese pavement, with a rapid decrease in particle size and alignment immediately afterwards (Fig. 2). The pattern of particle size and alignment is consistent with that predicted for a waning bottom current

Fig. 1 The magnetostratigraphy of cores CH115-62 and JC-GB-KS22 was determined from the inclination of the remanent magnetism after 100 oersted demagnetisation of each sample. The magnetostratigraphy is correlated with the polarity time scale¹⁶ using the first appearance of *Globorotalia truncatulinoides* (T) to determine the Plio-Pleistocene boundary and the last appearance of a late Pliocene fauna to determine the Matuyama-Gauss boundary¹⁷: *Globorotalia puncticulata* (P), *Globoquadrina altispara* (A), *Globoquadrina venezuelana* (V), *Globorotalia multicamerata* (M), and *Sphaeroidinellopsis seminulina* (S). Sedimentation resumed above the hiatus marked by the Mn-crust during the period 2.43-3.32 Myr. The low sedimentation rate in this section of the core precludes exact dating of the palaeomagnetic polarity log but we have tentatively placed the age of sediment above the crust at 2.90-2.94 Myr. If the short normal polarity event (2.90-2.94 Myr) is missing due to the sample interval, then the age of the crust is approximately 3.1 Myr. The age of the sediment older than 2.90 Myr in core JC-KS22 is based on an assumption of uninterrupted sedimentation below that level but the exact date cannot be determined due to the lack of foraminifera in that section of the core. The extinction of four index species (P, A, V, M) at the same level in core JC-KS22 may indicate an unconformity, however, the range of each species was determined by the presence of only a very few individuals so that the extinction may be an artefact of only a slight mixing.



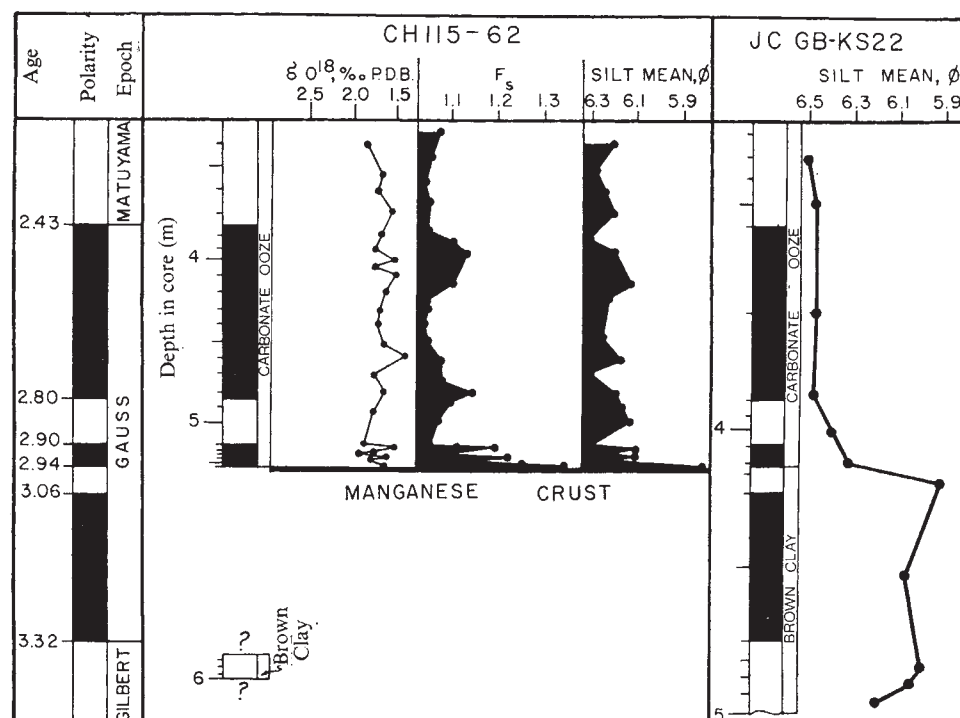


Fig. 2 The magnetostratigraphy of cores CH115-62 and JC GB-KS22 is used to date¹⁶ the $\delta^{18}\text{O}$ record of the planktonic foraminifer *Globorotalia inflata* (preparation as in ref. 17), the particle alignment analysis (anisotropy of magnetic susceptibility parameter F_s)¹⁸ and the silt mean particle size of carbonate-free sediment¹⁹. The manganese crust in core CH115-62 marks an erosional unconformity which has been correlated with a winnowed zone characterised by coarse silt mean values in the shallower core JC GB-KS22. The resumption of sedimentation above the unconformity is synchronous with a decrease in particle size in the winnowed zone. The $\delta^{18}\text{O}$ record shows a maximum shift of 0.5‰ during the time interval observed.

velocity which has fallen below a critical level for erosion so that only intense winnowing is observed²¹. Therefore, we ascribe the hiatus marked by the manganese crust to an increased bottom-water velocity event that decreased in intensity 2.90–2.94 Myr ago.

The hiatus in core CH115-62 prevents the dating of the initiation of the increase in bottom-water velocity. The particle size data in core JC GB-KS22, however, have recorded a winnowing event which began before 3.3 Myr and ceased at the time when sedimentation resumed above the manganese pavement in core CH115-62 (Fig. 2). Therefore, the high bottom-water velocity creating the scour zone was sufficiently reduced at the site of the shallower core (KS22) to produce only intense winnowing. The exact age of the initial increase in bottom-water velocity cannot be determined in core JC GB-KS22, as another hiatus is present below 500 cm (ref. 16) (Fig. 1). The apparent long duration of the increased velocity in the winnowing zone may be an artefact of a wide sampling interval in JC GB-KS22 which may have obscured fluctuations in bottom-water velocity of similar duration to those above the unconformity in core CH115-62.

If the production of AABW is coupled to global climate through any of the several mechanisms that have been proposed^{22–24}, then the initiation of the Northern Hemisphere ice sheet should be a dramatic test of the role of global climate on global abyssal circulation. Since the approximate 3 Myr age of the increase in bottom-water velocity in the Vema Channel is nearly synchronous with unconformities attributed to scour by AABW in both the Pacific⁵ and Indian⁶ oceans, the intensified bottom circulation in the late Pliocene may be considered as a global event. Therefore, the relative timing of the unconformities and the initiation of the Northern Hemisphere ice sheet is very important and may suggest a relationship between climate and abyssal circulation. The age of the buildup of the ice sheet, however, has been dated at 2.5 Myr (ref. 2) which is much later than the initiation of the increase in bottom-water velocity

(3.3 Myr) and is significantly later than the cessation of intense flow (2.9 Myr). To examine the relative timing of both events in the same core, oxygen isotopic analyses were performed on specimens of *Globorotalia inflata* from closely spaced samples from the late Pliocene section of core CH115-62 (Fig. 2). The greater than 1.2 per mil change in $\delta^{18}\text{O}$ recorded in earlier studies^{1,3,4} and interpreted as the initiation of the Northern Hemisphere ice sheet³ was not found in the Late Gauss or Early Matuyama portion of the core (Fig. 2) as was expected from the results of an earlier study on an exposed marine section in New Zealand².

The absence of the large change in $\delta^{18}\text{O}$ attributed to the initiation of the Northern Hemisphere ice sheet during the period in which it was previously described cannot be explained with the available data. Perhaps the single large shift in the $\delta^{18}\text{O}$ record observed in earlier studies using widely spaced samples^{1,3,4} may actually be a series of smaller changes which occur over a longer time interval than previously expected. The maximum 0.5 per mil change we observe may be one such small shift. Other late Pliocene sections must be analysed in high sedimentation rate cores using oxygen isotopic methods in combination with palaeomagnetic and biostratigraphic studies to determine the exact timing of the initiation of an ice sheet. If the results of those studies confirm the previously derived age of 2.5 Myr (ref. 2), then the increase in the production of AABW preceded the global cooling by at least 0.8 Myr and, therefore, could not have been caused by the global climatic change which produced the ice sheet. If the age of the ice sheet development is greater than 2.5 Myr, then a cause and effect relationship between climate and abyssal circulation may be examined in detail to determine which of the events triggered the other.

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Note added in proof: An age of 3.2 Myr has been assigned to the initial ice sheet development in a study using oxygen isotope analyses and magnetostratigraphy²⁵. The greater age of the ice sheet than was previously assigned^{2,3} may suggest that the climatic change responsible for the initiation was slightly preceded by the intensified abyssal circulation that we see just before 3.3 Myr (Fig. 2).

Table 1 Core parameters

Core	Latitude	Longitude	Water depth (m)	Core length (cm)
CH 115-62	30° 24.6' S	38° 58.4' W	4,065	712
JC GB-KS22	30° 30.0' S	38° 55.1' W	4,020	736

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Supernovae and lunar melting

THE view that a supernova explosion preceded the formation of the Solar System¹ by at most a few million years has gained support from the interpretation of meteoritic isotope anomalies by Latimer *et al.*² We suggest here another significant piece of evidence for this viewpoint, not mentioned in their report—that supernova-produced²⁶ Al (half life, 0.7 Myr) is also the only known physical heat source for melting of the lunar interior.

Libby *et al.*³ demonstrated that the Moon acquired a molten core on the basis of lunar figure, magnetism and other evidence. A review of possible heat sources for this melting argues that the only two reasonable sources were first, inclusion of radiogenic ²⁶Al within the Moon⁴ no more than a few million years from its formation in a supernova, and second, inclusion of a longer half life undiscovered 'superheavy' isotope. Gravitational energy seems to be quantitatively inadequate. Electrical heating⁵ requires particular assumptions about the nature of the interplanetary medium and the response of the Moon to hydromagnetic fluctuations. Collisional heating requires high velocity collisions, but remains a possibility.

Theoretical predictions of an 'island of stability' for transuranic elements make the second radioactive heating mechanism seem attractive. Evidence for the existence of such 'superheavy' elements has been tentatively claimed from interpretation of meteoritic xenon isotope distributions, but this interpretation is disputed (see ref. 6). Flerov *et al.* have reported indications of the presence of superheavy elements in the Allende meteorite⁷. In any case, the mere existence of such elements is insufficient to reconcile the melting of the Moon with the standard view of pre-Solar System nucleosynthesis occurring about 100 Myr before the origin of the Solar System. (The 100 Myr time scale is based on meteoric Xe, and to some extent on other isotope compositions⁸.) Further conditions

which must be satisfied are: (1) the existence of a superheavy isotope(s) with a half life near 100 Myr; (2) the existence of a chain of sufficiently stable intermediates for the formation of this particular superheavy isotope(s) during a supernova explosion; (3) formation of sufficient quantities to melt the Moon; and (4) penetration of this element into the protosolar cloud. Condition (1) is necessary because studies of lunar magnetic remanatism indicate that the lunar core was molten within a few 100 Myr of its formation², and if the half life of such an isotope is significantly less than 100 Myr, then ²⁶Al again becomes the preferred candidate. Conditions (2) and (3) are complex unresolved theoretical problems. Condition (4) can evidently be accomplished for ²⁶Al by penetration of the protosolar cloud by grains composed of known refractory minerals¹; a comparable chemistry might or might not exist for the hypothetical transuranic isotope. Collectively, these difficulties make it seem highly improbable that transuranic elements can explain the melting of the Moon. That leaves the only known available heat source as ²⁶Al.

The Moon need not actually have completely formed within a few million years after the supernova explosion which provided the ²⁶Al. But it is necessary that a substantial core had formed by that time and that the Moon continued to grow faster than heat diffused from the core. Theories of lunar formation are sufficiently vague to allow this possibility. Although it is easier to model lunar accumulation with a longer time scale, the only absolutely certain lower limit is the gravitational time scale, which is very small.

It therefore seems that lunar melting provides additional evidence for a supernova preceding formation of the Solar System by no more than a few million years. The existence of a supernova explosion a few million years or less before the formation of the Solar System does not preclude contributions to isotopic compositions from other previous supernovae, however. Allowance for this and for the possibility of formation of different Solar System objects such as the lunar core, meteorites, and lunar surface at somewhat different times may help reconcile the various time scales which date the origin of the Solar System.

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Opaque mineral alteration states correlated with temperature in an active geothermal system

ALTERATION of opaque minerals, especially the iron–titanium oxides, has become a very widely used technique in rock and palaeomagnetism and in geothermometry¹. The high ($\geq 600^\circ\text{C}$) temperature oxidation of titanomagnetite and ilmenite is a well-known process in nature^{2,3} and in experimental petrology^{4,5}. In contrast, the alteration of these oxides at low ($< 300^\circ\text{C}$) temperatures is relatively poorly known. This is partly due to the lack of data on the stability of various alteration products in the 0–300°C temperature range. Another difficulty is the absence of a characteristic sequence of textural features that can be used in a classification of progressive alteration. Here we describe how investigations on the Azores drill hole of 1973, located in the

lower flank of the active volcano Agua de Pau, San Miguel, Azores⁶ have filled this gap.

Bottom hole temperatures were measured during and after drilling and exceeded 200 °C from 500 m to 900 m depth⁶. The temperatures of equilibration of vug and vein calcite with water were calculated from the oxygen and carbon isotopic ratios (J. R. Lawrence and S. Maxwell, in preparation). Results matched the observed high temperatures over several sections of the drill core. The X-ray study of secondary minerals (P. K. Sarkar unpublished data) suggests that the composition and nature of the fluids have changed from acidic (rich in HCl) to neutral and alkaline as a result of leaching of the country rocks. Examination of 232 polished sections representing the lava flows and explosive rock types of the drill hole section has shown that a wide range of degrees of hydrothermal alteration is present. The alteration states of titanomagnetite and ilmenite were identified microscopically and the chemical compositions were determined using an electron probe microanalyser. The correlation between the average measured and calculated temperatures and the titanomagnetite and ilmenite alteration states are given in Fig. 1 and 2. Visible alteration of titanomagnetite starts at a temperature of 40 °C with the appearance of randomly distributed whitish-blue irregular lines of titanomaghemite. These lines become thickened and join each other to form patches of titanomaghemite that increase in area with increasing temperature. At a temperature of ~ 120 °C, titanohematite begins to replace titanomaghemite. This replacement continues until a temperature of 150 °C is reached. At this temperature titanohematite becomes unstable and transforms to a granular phase rich in TiO₂. The granules have a variety of colours (blue, orange, yellow and red), they spread in titanomagnetite grains and continue to increase in area until a temperature of ~ 180 °C is reached. At this temperature, sphene and limonite begin to form.

Visible alteration of ilmenite starts at a temperature of 80 °C with the formation of ilmenite type (2) which is less pinkish in colour and has lower anisotropy than the original ilmenite. At a temperature of about 90 °C rims, patches and irregular lamellae of light grey pseudorutile⁷ (ideally Fe₂Ti₃O₉) appear. Pseudorutile continues to replace ilmenite (2) but at about 110 °C, begins itself to transform to hematite and rutile. A granular phase rich in TiO₂ starts to form at 150 °C and increases in area with increasing temperature to about 170 °C. At this temperature white leucosene, associated with a yellow sphene appear. Finally, sphene and limonite form at a temperature of about 200 °C.

Although there is uncertainty in the nature of granular phases in both titanomagnetite and ilmenite, they are always rich in TiO₂ and start to appear only at temperatures of 150 °C and

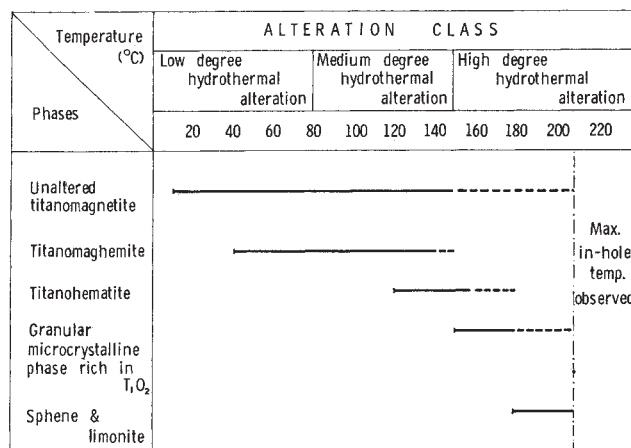


Fig. 1 The temperature intervals over which titanomagnetite and its alteration products occur in the Azores drill hole. Solid line, present as a major phase; dashed line, present as a minor phase.

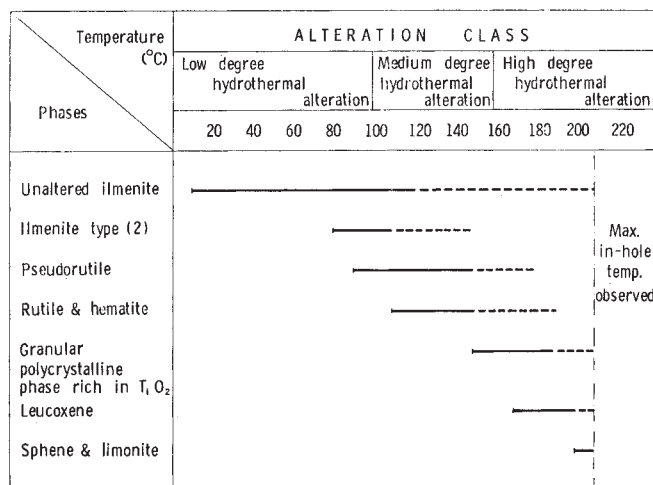


Fig. 2 The temperature intervals over which ilmenite and its alteration products occur in the Azores drill hole. Solid line, present as a major phase; dashed line, present as a minor phase.

higher. The fine crystallinity of the granular phases make their X-ray analysis rather difficult (D. Plasse, personal communication). These temperature stability fields will be of interest in experimental work on oxides and in mapping palaeoisotherms.

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Were Archean continental geothermal gradients much steeper than those of today?

ARCHEAN geothermal gradients have often been supposed to be much steeper than those of today¹⁻⁶. Because heat generation from the decay of radioactive nuclides in the Earth was then much greater^{4,7,8}, extra heat, if it escaped by conduction, must have been carried along steeper thermal gradients. Significant measurements of terrestrial conductive thermal gradients are very difficult to obtain, and estimates of thermal gradients in the past are even harder to make. Estimates made from igneous and metamorphic rock occurrences are of limited value both because igneous rocks occur at levels in the earth shallower by unknown amounts than those at which they form and because nearly all igneous and metamorphic rocks are formed at plate boundaries where thermal gradients are much steeper than within plates. In plate margin areas, advective and convective processes dominate so that the normal conductive gradient of

the lithosphere can only be measured or estimated in areas away from plate boundaries. We show here that the rocks of the Superior Province (formed roughly in the interval 3,100–2,500 Myr ago) were not subjected to steep regional geothermal gradients after their assembly into a continent by lateral accretion of island arcs⁸, and hence that the extra Archean heat escaped the Earth in some way other than by conduction through the continent. We suggest that this escape was mainly by cooling of the ocean floor as a boundary conduction layer.

The Superior Province⁹ consists of greenstone belts, the rocks of which have been compared to rocks formed in various island arc and marginal basin environments⁸ and intervening gneiss belts that have been compared to more deeply eroded arcs or microcontinents⁸. Although the gneiss belts show great structural complexity and may include some high-grade metasediments granodioritic and tonalitic compositions greatly predominate. To a first approximation, the gneissic terrains can be regarded as of a granodiorite–tonalite average composition⁹. Wise has shown by considering the control on continental thickness exercised by the depth of water in the ocean¹⁰ that continents 2,500 Myr ago were about as thick as they are now. Seismic refraction measurements¹¹ in the Superior Province indicate a depth to the base of the crust of about 35 km.

Rocks of granodioritic composition subjected to pressures appropriate to a depth of 35 km (about 10 kbar) begin to melt at a temperature close to 700 °C leaving a dry and refractory residuum (see, for example, Fig. 1b in ref. 12).

The products of melting which would tend to rise buoyantly in the crust and thus to become exposed by erosion at the surface are typically granites of minimum-melting composition (roughly equal amounts of quartz, albite and potash feldspar). Such granites are abundant in many crystalline terrains, for example: the Grenville, Pan-African and Churchill provinces and have been compared to the acid volcanics of Tibet produced by partial melting of thickened continent¹³. Minimum-melting granites are extremely rare in the Superior Province, and we interpret this scarcity as an indication that the temperature 35 km down at the base of Superior Province crust in Archean time did not generally exceed 800 °C. We reject the idea that minimum-melting granites may have occurred at higher levels now eroded from above the Superior Province, because the preservation of low-grade assemblages in the greenstone belts of the province precludes deep burial and because of the limited occurrence of granulitic rocks in the province. As the temperature at the base of the continental crust today is commonly estimated¹⁴ to be about 500 °C, we conclude that in Archean times a typical continental geothermal gradient was less than 23 °C km⁻¹ compared with 17 °C km⁻¹ today. Because heat generation rates in the Earth between 2,500 Myr and 3,100 Myr ago may have been as much as three times greater than at present⁷, the possible difference of 30% in the slope of the continental geotherm is insufficient to remove all the extra heat then being generated.

How might the extra heat have left the Earth? Consider how heat leaves the Earth's surface today, escaping from the solid earth in three ways which each account for roughly one-third of the total: by conduction through the continents; by conduction through the ocean floor and by making and ageing oceanic lithosphere as a boundary conduction layer to mantle convection. An accurate assessment of the proportions of heat removed by these three processes is not possible, but it is suggested (J. D. Sclater, personal communication) that one-third may be too low an estimate for making and ageing oceanic lithosphere. During the Archean, the two conduction processes could have accounted for more heat, as both the mantle and the overlying continental and oceanic crust would have contained more heat generating nuclides, but the evidence from the Superior Province (most other Archean terrains are similar) shows that increased conduction through the continents was not significant. Although conduction through the largely unpreserved Archean ocean floor may have been greater than at present, another way of removing very much more heat

from the Earth is by making and cooling extra ocean floor. This could have been done by having a greater total length of ridge in the Archean ocean or by spreading and thus making ocean floor faster at ridges, or by both processes. Since the rate at which ocean floor cools decreases as a function of age, more heat is removed from the Earth if the average age at which the ocean floor is subducted is reduced. Ultramafic komatiites¹⁵, rocks that may represent a much larger proportion of partial melting of the mantle than normal basalts, are apparently almost restricted to the Archean and have sometimes been considered as evidence for very steep Archean thermal gradients⁸. These rocks occur as a small proportion of piles of basalts very similar to mid-ocean ridge basalts and we follow the suggestion¹⁶ that ultramafic komatiites are melts from great depths that have risen diapirically to the surface and escaped the near surface equilibration that is represented by basalt. We associate this process with the more intense convective circulation that we believe characterised the Archean rather than with a steep conductive oceanic thermal gradient.

Some have doubted whether plate-tectonic processes operated in the Archean, but the absence of granite from the Superior Province indicates that the great heat of the ancient Earth was not removed by conduction through continental crust. A convective process seems necessary to dissipate this heat and plate-tectonics is an efficient and familiar convective process capable of doing the job.

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Induced suction and lithospheric plate pull

GEOPHYSICISTS have not agreed on the source of the driving force which propels the Earth's lithospheric plates over the asthenosphere. Three principal sources have been investigated: first, that somehow the downward pull exerted upon the floating lithosphere by the subduction process at oceanic trenches is translated into a horizontal driving force^{1–3}, second, that the process is one of downhill sliding from elevated mid-ocean ridges to depressed trenches^{4–8} and third, that mantle convection currents are responsible^{9–11}. It is agreed, however, that whatever the origin of the driving force, it must be of the order of 10¹⁵ dyn cm⁻¹ (refs 2, 12–14) (per unit distance along the plate surface normal to the force) and must, of course, have a direction consistent with present and past kinematical patterns. We show here that a phenomenon which we term 'induced suction' can account for the driving force. Fortunately, it can be investigated fairly rigorously by simple principles of statics. (The term suction has been previously applied to an unrelated driving force phenomenon)^{14,15}.

A well known principle of fluid statics is that an object of arbitrary shape immersed in a fluid at rest has zero net horizontal force exerted on it by the fluid, and this is true if the fluid density depends on vertical position. It is not true, in general, if the density is horizontally dependent (a case which is not normally encountered as the resulting horizontal pressure gradient will eliminate such a density variation). This anomalous situation is created, however, by the subduction process—the sinking of an oceanic plate into the mantle at a trench (Fig. 1). Thus, the lithosphere with a density slightly greater than that of the supporting asthenosphere pulls downward with force F_V . The depressed triangular regions GHAG and HBAH are filled with water and deformed sediments with densities of 1.05 and about 2.4 g cm⁻³, respectively. On the other hand, at the opposite ridge side of the oceanic plate, the plate is entirely bounded by high density material of about 3.35 g cm⁻³. The result is an induced horizontal plate force which one could term either a ridge push or trench pull. Because the origin of the force is the 'suction' by the low density trench material we shall term it a 'pull'.

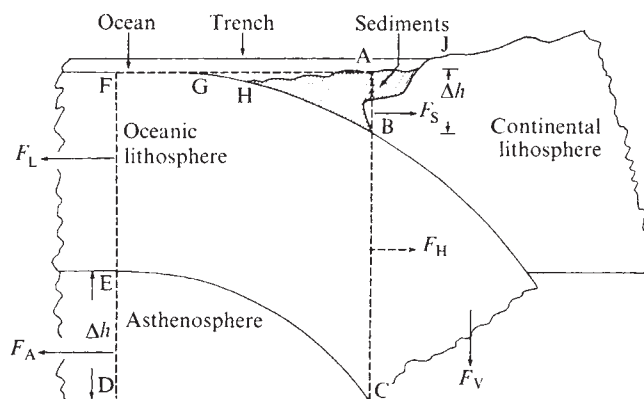


Fig. 1 A section of lithosphere normal to the trench axis where bending occurs and subduction is initiated. Region HBJAH is accreted sediment, $\rho_s \sim 2.4$ g cm⁻³, and region AGHA water, $\rho_w = 1.05$ g cm⁻³. The vertical exaggeration makes it appear that DE and AB are not equal.

The force is actually generated in a narrow region bordering the trench where bending occurs. In the analysis we choose a rectangular section ACDFA, where side AC corresponds to the greatest depth of sediment and where side CD is long enough to include the plate bending region to the left of BC. We consider the horizontal equilibrium of the rectangular section where we neglect intraplate forces and mantle interactive forces. (We are interested here only in the plate force which suction creates. Any forces which we now neglect would simply be added in the final force tabulation so that these assumptions are not really restrictive.) In Fig. 1, sides EF and BC are bounded by material of density $\rho_L = 3.4$ and sides AB and DE by material of densities $\rho_s = 2.4$ and $\rho_A = 3.35$, respectively. For convenience we subtract ρ_L from these values so that the hydrostatic stress due to the lithospheric weight is subtracted from all calculations. In Fig. 1 the rectangle is now bounded on BC and EF by material of density zero and on AB and DE by material of densities $-(\rho_L - \rho_s)$ and $-(\rho_L - \rho_A)$, respectively.

We now make two assumptions. First, hydrostatic stress conditions apply in the accreted sedimentary prism HBAH and on the asthenospheric face DE. This really means that the materials in these regions behave as fluids and that shear stresses are negligible. Second, the vertical loading $-(\rho_L - \rho_s)g\Delta h$ which the sedimentary prism exerts on the lithosphere near point B does not induce any net horizontal force F_H (Fig. 1). (This agrees with the common experience that one cannot propel a solid plate horizontally by pulling on it vertically.)

Thus the net force on BC vanishes, whereas on AB

$$F_s = (\rho_L - \rho_s)g \int_0^{\Delta h} z \, dz = (\rho_L - \rho_s)g(\Delta h)^2/2 \quad (1)$$

and similarly on EF

$$F_A = (\rho_L - \rho_A)g(\Delta h)^2/2 \quad (2)$$

As F_A is only 5% of F_s it will be neglected. Consideration of the horizontal equilibrium of rectangle ACDFA shows that a lithospheric tensile force F_L is 'induced' to balance the suction F_s . Hence,

$$F_L = (\rho_L - \rho_s)g(\Delta h)^2/2 \quad (3)$$

Data on sediment depths Δh are scanty and probably imprecise. Values in the range 10–26 km are indicated for most trenches^{16–18}. When substituted into equation (3), this yields a range for F_L between 0.5×10^{15} and 3.4×10^{15} dyn cm⁻¹. (Some trenches have shallow accretionary prisms. In these cases, if equation (1) is calculated at a vertical section through the trench H (Fig. 1) values of F_L of the order $1-3 \times 10^{14}$ dyn cm⁻¹ are obtained.)

By this model the pressure at point D is less than that at C by the amount $(\rho_L - \rho_A)g\Delta h$. Thus our estimate of the magnitude of F_C should be conservative; if we had made assumptions which resulted in equal pressures at points D and C, values greater than those produced by equation (3) would result.

The model leads to the following conclusions. First, induced suction generates the right order of magnitude of lithospheric force. Second, the model predicts that for two similar plates (differing only in size) the net driving force or torque depends linearly on a characteristic surface dimension, whereas basal shear resistance depends on the square of the dimension; if other things are equal, small plates should move faster than large ones. Finally, the same force F_L is induced on the overriding plate, which could account for certain long time creep induced phenomena such as back-arc spreading, the stretching of continental plates behind island arcs.

The model does not answer the question of how the subduction process originates and it does not rule out other origins of plate motion. The Antarctic plate, for example, which is bounded by ridges, is not affected by induced suction. It could be moved by ridge push, convection currents, or by interplate forces.

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Soils on Santorini at ~1500 BC

THE discovery and excavation of the Minoan settlement near Akrotiri, Santorini (Thera) was the outstanding achievement of the late Sp. Marinatos¹. Life in the town came to an abrupt end at ~1500 BC with the volcanic eruption which was preceded by a major earthquake². An interesting palaeoenvironmental problem is the nature of the land resource base which supported such a culturally rich society. One key component of the physical environment would have been the soils as they existed in Minoan times. The usual situation in palaeopedological investigations is the lack of buried soils, but the distinctive geological evolution of Santorini has meant that the pumice and tephra of the ~1500 BC eruption sealed the late Minoan landscape. We report here some results which describe the material immediately below the pumice. It is suggested that the Minoan soils like those of today were poorly developed, suffered from erosion and posed fertility problems to the Minoan farmers.

Luce³ describes the Upper Pumice Series dating to the Minoan eruption which directly overlies a palaeosol and more recently Pichler and Friedlich⁴ state that this old soil reaches a thickness locally of 3–4 m. A period of ~15,000 yr without any volcanic activity on Santorini preceded the Minoan eruption and during this time the underlying ignimbrites were decomposed and altered to a thick palaeosol⁴. The aim of the present study was to examine the morphological evidence for these palaeosols supplemented with laboratory analysis of soil samples. It was assumed that former soil horizons could be recognised in the immediate sub-pumice level; it was also hypothesised that former pedogenesis would be reflected in the presence of organic matter and its vertical variation.

The thickness of the Upper Series varies over the island; in the Phira quarries in the central part of the island these deposits have a maximum depth of about 30 m. Such a thickness of pumice and tephra posed serious problems for the location of sites which exhibited the supposed palaeosol immediately below the diagnostic fine pellety pumice. However, six appropriate exposures were identified mainly in the southern part of the island and their locations are shown on Fig. 1. Exposures of the same stratigraphic sequence were also sought in the east-central part of Santorini, but without success. The only other types of sites encountered were on steep hillslopes; for example, on the east side of Mesa Vouno, pumice presumably from the Minoan eruption, directly overlies in places either the bedrock of marbles and phyllitic schists or a rubbly drift derived from these rocks. The implication is that the surface of these steep hillslopes in Minoan times was characterised either by the absence of soil or by the presence of a thin, rubble-derived soil.

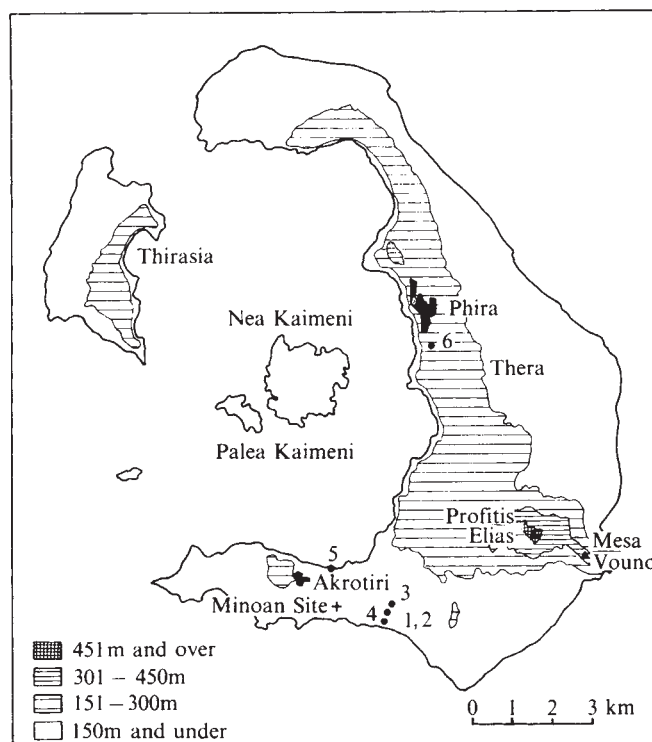


Fig. 1 Santorini (Thera) and the location of the sites where the Minoan soil was sampled. Exposures 1, 2 and 3: sections at base of ravines in the deeply eroded area to the east of the archaeological site near Akrotiri. Exposure 4: section near base of cliff on coast to the east of the archaeological site. Exposure 5: section at side of footpath down to the small harbour to the immediate north of the village of Akrotiri. Exposure 6: section near base of Phira quarry to the immediate south of Phira.

The material immediately below the fine pellety layer of pumice was carefully examined in the field in order to establish if any former soil horizons could be identified. In most cases colour differentiation was not apparent, although a slight contrast was evident in exposure 3 (Table 1). This suggests that there are no distinct buried soil profiles. In addition the material appeared loose and sandy, often with a significant amount of gravel. To obtain further evidence about the Minoan soil, samples from the exposures were analysed for particle size⁵, organic matter⁶ and cation exchange capacity⁷, and the results are given in Table 1. Two samples of present-day topsoil derived from tephra are included for comparison.

Table 1 Soil analytical data

Exposure	Sampling depth (cm)	Colour	>2,000	Particle size (μm) (2,000–63) sand	(63–2) silt	(<2) clay	Organic matter % of < 2 mm	Cation exchange capacity m.e. per 100 g
1	5	7.5YR3/2	41	49	9	1	0.1	5
2	5	7.5YR3/2	16	71	12	2	0.1	6
3	5	10YR4/3	23	63	12	2	0.4	7
3	13	10YR4/2	20	65	13	2	0.1	8
4	5	7.5YR3/2	34	62	3	1	0.1	5
5	5	7.5YR3/2	18	72	9	1	0.1	5
5	38	7.5YR3/2	16	74	9	2	0.1	6
6	5	10YR5/3	22	66	11	1	0.1	9
6	38	10YR4/3	5	83	12	1	0.1	10
7	—	2.5Y5/2	37	60	3	1	0.1	9
8	—	2.5Y5/6	24	58	16	3	0.3	3

Exposures 1–6 inclusive refer to material from the Minoan level whilst exposures 7 and 8 are from present tephra-derived soils. The sampling depth is measured from the base of the fine pellety pumice.

The outstanding feature about all the particle size data is the coarse nature of the material—predominantly sands with only small quantities of silt and almost no clay. The organic content is negligible; the only suggestion of a former horizon with some organic matter is at exposure 3. The organic content figures should be seen in relation to the very slight amounts for present soils. Cation exchange capacity is a useful general indicator of soil fertility and, again, the results for both the Minoan and present soils are low.

Evidence for well-developed soils on Santorini at the time of the eruption cannot be presented. Instead the evidence at best suggests that only poorly developed soils existed in the lower areas in what is now central and southern Santorini. On the steep slopes of the higher areas, there would have been either bare rock surfaces or poorly developed soils on rock rubble. The striking characteristic of the results in Table 1 is the similarity in terms of particle size, cation exchange capacity and organic matter between these Bronze Age soils and the present ones.

The inference is that the long period of geological stability before 1500 BC is not indicated in the soil conditions at the time of the eruption. Remains of trees have been reported from the Minoan level³ as well as carbonised plants from a lower level of the palaeosol in Therasia⁴; such evidence raises the possibility that better soil conditions could have existed before 1500 BC. If these soils had existed, however, they would have been subjected to major erosion and general deterioration by the time of the eruption. Such erosion is postulated on the basis of the similarity between the tephra derived soils of today and the Minoan soils developed on the ignimbrites. The efficiency of erosional processes is also indicated by the irregular nature of the unconformity at the base of the fine pellet pumice as exposed in the extensive Phira quarries. Soil erosion is very apparent on Santorini today, reflected most clearly in the stone-dominated nature of the surface and the corresponding deposition of finer material against walls. Despite the 3,500 yr since the cataclysm, soils are poorly developed and their character is largely a result of the erosional processes, underlying geology and Mediterranean climate. An additional factor, which must have contributed to soil instability immediately before the eruption, was the occurrence of earthquakes. This evidence of active soil degradation on Santorini at 1500 BC is in accord with results from Melos where recent investigations have suggested that accelerated erosion was underway by 1000 BC or soon afterwards⁵. At least latterly the Minoan farmers would, like their present counterparts, have had to contend with serious soil moisture deficits, a soil low in organic matter and clay resulting in poor water and nutrient storage, as well as a soil highly susceptible to erosion.

I thank the Carnegie Trust and the University of Strathclyde for grants towards the cost of fieldwork, also Dr C. Doumas for assistance with my visit. Mrs L. Nelson analysed the samples and Dr K. Smith made helpful comments. Full details of the work on Santorini are to be presented at the Second International Scientific Congress on Thera and the Aegean World.

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Marine alga *Platymonas* sp. accumulates silicon without apparent requirement

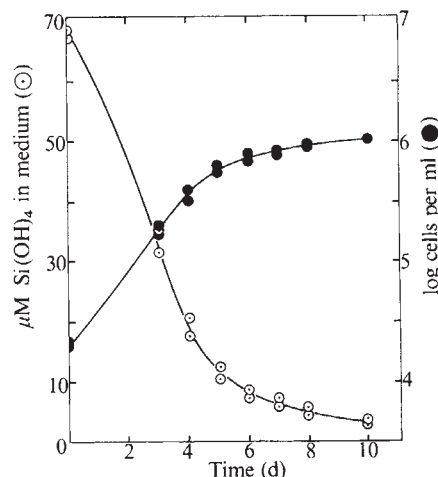
THE role of silicon in living systems has only recently begun to be understood. Apart from being an important structural component of diatoms and silicoflagellates¹, some freshwater flagellates², higher plants, sponges and other organisms³, it is necessary for DNA polymerase activity in diatoms⁴ and for bone and tooth development in certain vertebrates⁵. Silicified algal species are known (or assumed) to have an obligate, concentration-dependent silicon requirement for growth^{6,2}, and transport systems for the accumulation of silicic acid ($\text{Si}(\text{OH})_4$) have been described⁷; however, the uptake of $\text{Si}(\text{OH})_4$ by nonsilicified algae has never been reported. Here we report the accumulation of significant amounts of silicon by the planktonic marine alga *Platymonas* sp. (isolated during the summer of 1954 from Great Pond, Falmouth, Massachusetts). This alga is very close to, or may be an isolate of *P. suecica* (personal communication from R. Norris) and has no known silicon requirement, growing equally well in Si-rich and Si-poor environments. Because $\text{Si}(\text{OH})_4$ is a potentially limiting nutrient for diatoms⁸, the removal of available $\text{Si}(\text{OH})_4$ from the environment by organisms that do not require it could play an important role in phytoplankton competition and succession. This phenomenon could also be important in the cycling of silicon in the ocean.

Our investigation was stimulated by the discovery that certain species of marine phytoplankton, particularly members of the Dinophyceae, Prasinophyceae, Prymnesiophyceae and Cyanophyceae contained amounts of silicon equivalent to that found in diatoms of comparable size, while other species contained no silicon although grown in identical conditions (unpublished data of R.R.L.G., N. S. Fisher, G. Thompson and D. C. Bankston). One of the Si-containing algae, *Platymonas* sp. (clone WHOI 'Platy 1'), was chosen for further study because it grows rapidly and attains high densities.

Platymonas sp. cultures were grown under continuous light (Sylvania 'cool-white') of 10 klux at 20 °C in polycarbonate Erlenmeyer flasks containing the artificial seawater base known as SOW (ref. 9) enriched with nitrate, phosphate, vitamins and trace metals as in half-strength medium f (ref. 10). The concentration of silicic acid varied as specified.

Uptake of $\text{Si}(\text{OH})_4$ by *Platymonas* sp. was monitored by measuring the change in $\text{Si}(\text{OH})_4$ concentration in batch cultures. The cultures were inoculated with a small inoculum (about 10^4 cells) from a culture that had been grown without added silicon. At various stages during the growth of the culture,

Fig. 1 Time course of cell density and silicic acid concentration in duplicate batch cultures of *Platymonas* sp.



duplicate samples were removed, filtered through either Millipore or Nucleopore filters (0.8- μ m and 1.0- μ m pore diameter, respectively) and the filtrate was analysed colorimetrically for reactive silicate¹¹. Cell density was measured both microscopically, using a haemocytometer, and with an electronic particle counter (Coulter Electronics).

Platymonas sp. removed significant amounts of Si(OH)_4 from the growth medium during exponential growth and, to a lesser extent, during early stationary phase (Fig. 1). Control experiments showed no change in Si(OH)_4 concentration during this interval in flasks not inoculated with cells. Experiments involving different initial Si(OH)_4 concentrations and inoculum sizes yielded similar patterns, with uptake apparently related to growth rate and/or Si(OH)_4 concentration in the medium. The latter can be seen more clearly when the data from two experiments with different initial Si(OH)_4 concentrations (164 μ M and 68 μ M are replotted as in Fig. 2. Here the change in Si(OH)_4 concentration divided by the concurrent change in cell number for a given sampling interval is plotted against the average Si(OH)_4 concentration over that interval. The relationship found can be approximated by a line:

$$-\Delta[\text{Si}]/\Delta N = M[\bar{\text{Si}}] \quad (1)$$

where M is a constant, $[\text{Si}]$ is the Si(OH)_4 concentration in the medium, N is the cell concentration, and $[\bar{\text{Si}}]$ is the average Si(OH)_4 concentration during a given sampling interval. If both sides of this equation are multiplied by the instantaneous growth rate $(1/N) \cdot (\Delta N/\Delta t)$, we obtain:

$$-\Delta[\text{Si}]/(N\Delta t) = M[\bar{\text{Si}}] \cdot \Delta N/(N\Delta t) \quad (2)$$

The left side of this expression represents the rate of Si(OH)_4 uptake per cell which is shown to be proportional to the growth rate of the culture and the Si(OH)_4 concentration in the medium, at least for the concentrations examined.

To confirm that the disappearance of Si(OH)_4 from the growth medium was due to cellular uptake, and to characterise in part the intracellular fate of the Si(OH)_4 , *Platymonas* sp. cells were analysed for both total intracellular silicate and water-soluble intracellular silicate at the end of growth in media containing silicic acid. The Si(OH)_4 that disappeared from the medium was essentially 100% retrievable in the cells (Table 1). Roughly 30% of the intracellular silicate was water soluble and the total amount of silicate measured per cell was 8×10^{-8} μ mol. For all the cultures analysed by either the disappearance or direct measurement method, the silicon per cell value ranged from 6×10^{-8} to 11×10^{-8} μ mol, which is close to values reported for diatoms of similar size⁶. Given an average cell volume of 180 μm^3 (as measured in an electronic particle counter) for *Platymonas* sp., intracellular concentrations of total Si(OH)_4 acid could be as high as 500 mM with more than 100 mM Si(OH)_4 in the dissolved fraction. This high value for the dissolved fraction probably means that silicon exists in the form of short polymers, because monosilicic acid should autopolymerise near 3 mM Si(OH)_4 (ref. 12).

Although this evidence clearly demonstrates that *Platymonas* sp. can take up significant quantities of Si(OH)_4 , we were

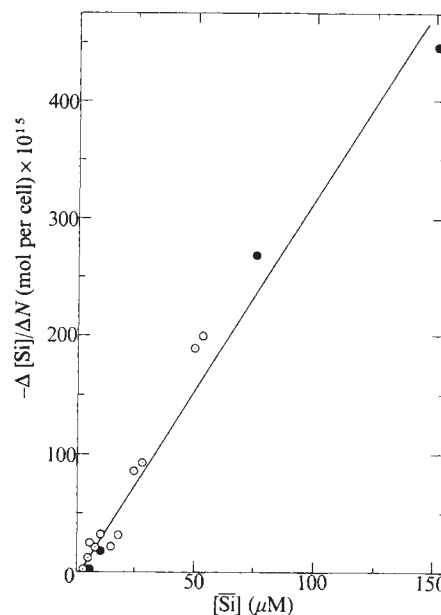


Fig. 2 Ratio of silicic acid taken up to increase in cell number plotted against mean silicic acid concentration during the growth cycle of *Platymonas* sp. in batch culture. ○, Data from the experiment shown in Fig. 1; ●, from a similar experiment in which initial Si(OH)_4 concentration was 164 μ M.

unable to demonstrate a silicon requirement in this alga. Cultures grown with 0, 1 or 100 μ M added Si(OH)_4 in polycarbonate flasks had indistinguishable growth rates and final yields. Because a small amount of silicon (about 1 μ M) contaminated the medium we cannot rule out the possibility that *Platymonas* sp. has a microrequirement for silicon. Although this genus has scales on its body and flagella¹³, they have not been shown to contain silicon.

There are strong indications that many other species of marine phytoplankton (with no known Si-requirement) can contain significant quantities of silicon (R.R.L.G. *et al.*, unpublished data), and during the study reported here, we observed the disappearance of Si(OH)_4 from the growth medium of the freshwater species *Euglena gracilis* (Z). Also *Phaeodactylum tricornutum*, an atypical marine diatom lacking organised silicified structures and without demonstrated Si-requirement can accumulate up to 10^{-8} μ mol Si(OH)_4 per cell (D'Elia *et al.*, unpublished data). Such uptake of significant quantities of Si(OH)_4 by organisms that do not require it could be of ecological importance. Because natural waters are often low in Si(OH)_4 relative to the requirements of diatoms, uptake of this element by organisms such as *Platymonas* sp. could represent a mechanism by which they limit the growth of other species without affecting themselves, a relationship known as amensalism. Substantiation of this hypothesis awaits the demonstration that these species have high affinity Si-transport mechanisms, similar to that of diatoms, capable of

Table 1 Partitioning of silicate at stationary phase in two batch cultures of *Platymonas* sp.

Si(OH)_4 disappearing from medium (μ M)	Total intracellular silicate (μ M)	Soluble intracellular silicate (μ M)	% Soluble intracellular silicate	Stationary phase cell concentration (cells per ml)	Cellular silicate at stationary phase (μ mol per cell)
67.3	69.1	16.2	23	8.5×10^5	8.1×10^{-8}
63.5	62.9	22.1	35	7.4×10^5	8.5×10^{-8}

Total intracellular silicate was measured by a modification of the base hydrolysis method of Paasche⁶. Duplicate 18-ml samples were centrifuged in plastic tubes (1,600g for 10 min) and the pellet was resuspended in 15 ml of 1N NaOH and placed in a boiling water bath for 20 min. The hydrolysate was then cooled and neutralised with 3 ml of 5N HCl, filtered and analysed for reactive silicate. Water-soluble silicate was measured by a distilled water extraction. Duplicate 18-ml samples were centrifuged (1,600g for 10 min) and the cells were resuspended in distilled water and lysed by sonication. The lysate was filtered and analysed for reactive silicate.

assimilating $\text{Si}(\text{OH})_4$ when it is present in low concentrations.

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Limb morphology and function are transformed by contralateral nerve section in snapping shrimps

PERIPHERAL nerves exert strong trophic influences on the muscles they supply. Although not well understood, the crucial role of nerves in regulating development, regeneration and normal maintenance of limb muscles in vertebrate and invertebrate animals is assumed to depend on a direct anatomical relationship between the axons and their targets. The trophic nervous mechanisms which operate during limb regeneration and development may be studied profitably in crustaceans, many of which possess opposite limbs which are morphologically and functionally dissimilar. Homarid lobsters, for example, have one enlarged heavy crusher claw and a more slender, highly serrated ripper claw on the opposite side. Sexually mature male fiddler crabs have a greatly enlarged claw on one side only, and many other crustaceans show similar bilateral differences in relative claw size. The snapping shrimps (Family Alpheidae) show especially dramatic cheliped asymmetries. In these animals, one of the two claws is tremendously enlarged

and also differs greatly in external morphology from its opposite number (Fig. 1). Snapping shrimps use their enlarged 'snapper' claw mainly in aggressive encounters with conspecific shrimp¹. Exoskeletal and/or muscular arrangements permit the dactyl of the snapper claw to close against the propodite with great force². This action generates an intense jet of water from a specialised region of the propodite and also produces an audible snap. We describe here changes not only in the size and function, but also in the morphology of the terminal limb segments in this shrimp following damage to a distant anatomically separate nervous pathway. At least one, if not several, synapses intervene between the damaged neurones and those which make functional contact with muscles in the affected limb. Thus, these findings imply that peripheral nerves need not make direct anatomical contact with the targets of their trophic influences.

In snapping shrimps, as in other crustaceans, lost appendages are eventually replaced, although, as originally observed by Przibram³ and Wilson⁴, the nature of the regenerated appendage



Fig. 1 *Alpheus armillatus*, from dorsal view. The large right-hand cheliped is the snapper.

is not necessarily identical with the original amputated member. A new pincer claw will simply regenerate if the original pincer is lost through autotomy or amputation. However, loss of the snapping claw induces a dramatic transformation of the remaining pincer into a snapper, while a new pincer regenerates at the site of the original snapper. The pincer-snapper transformation, a reversal of the original asymmetry, occurs within two moults following snapper loss. After the first moult, the transforming limb is intermediate in appearance between pincer and snapper. At the second moult transformation to snapper is complete, at least in the three species of shrimp that we have studied: *Alpheus heterochelis*, *Alpheus californiensis* and *Alpheus armillatus*.

Table 1 shows results of claw amputation in two species of animals. Figures in the left-hand column show numbers of living animals at least two moults following the indicated

Table 1 Results of amputation in two species of animals

No. of animals	Original configuration				Limb removed		Final configuration			
	Snapper		Pincer		Snapper	Pincer	Snapper		Pincer	
	Left	Right	Left	Right			Left	Right	Left	Right
<i>A. heterochelis</i>										
18	×			×	×			×	×	
20		×	×		×		×			×
7		×	×		×	×		×	×	
5	×			×	×	×	×			×
<i>A. californiensis</i>										
3		×	×		×		×			×
4	×			×	×			×	×	
5	×			×		×	×			×
3		×	×			×		×	×	
2		×	×		×	×		×	×	
1	×			×	×	×	×			×

Table 2 Results of severing both nerves supplying a snapper cheliped

Group	No. of animals	Died	Lost or autotomised affected limb	Pincer transformed after two moults
Experiment	70	18	47	5
Control	24	4	2	0

operations. Removal at the autotomy plane of both claws or the pincer claw alone results in retention of the morphological *status quo*, whereas snapper removal produces morphological and functional transformation of the pincer. Thus, either side is capable of developing both types of claw. Why is it that pairs of snapper or pincer claws are never observed in the same animal, and what accounts for the transformation of the pincer to the snapper following loss of the latter? On a superficial level, a number of different schemes could account for the experimental results in Table 1, but we will consider only two especially compelling possibilities.

Assume that a message, generated during amputation of either claw by damage to claw tissue or nerve supply induces snapper transformation of the contralateral appendage, and pincer regeneration of the ipsilateral limb. This scheme could account for the results of unilateral amputation of either claw: snapper amputation results in the opposite claw being transformed from pincer to snapper, while the ipsilateral stump becomes a new pincer. Pincer amputation maintains the opposite snapper claw while, again, the ipsilateral stump regenerates into a new pincer. To explain the maintenance of the *status quo* that characterises the regeneration of bilateral amputees, however, an additional postulate is required, perhaps one which invokes cancellation of influences from the opposite side as a consequence of bilateral amputation. The reappearance of the original claw asymmetry might then result either from genetic or nervous organisational bias.

A different control hypothesis, one originally proposed by Wilson⁴ and involving fewer *a priori* assumptions, can be postulated to fit the observations of Table 1. In this scheme, a pincer is regarded as an immature snapper, its further development being suppressed by the presence of the contralateral snapper. Pincer amputation alone thus leads to regeneration of another suppressed claw—a pincer. Snapper amputation releases the remaining pincer from suppression, and its completed development follows during the succeeding two moults. In consequence, the new regenerate on the opposite side is a pincer. Retention of the *status quo* following bilateral amputation is harder to explain, but it could result from bias, as discussed above.

If suppression operates to assure the presence of a single snapper claw per animal, what is the nature of the message, where is the message elaborated, and how is it transmitted? If the site of origin or the transmission pathway of the message is removed without the concomitant loss of the snapper claw itself, can animals be produced which possess snappers on both sides?

From studies with other arthropods, it is well known that motor nerves exert a necessary trophic action on the normal development of skeletal muscles⁶⁻⁷. We were interested in the possibility that neuronal influences may constitute the message which controls claw morphology in the snapping shrimp (*A. heterochelis* and *A. armillatus*). In fact, Wilson's original work⁴ showed some influence of the limb nerves in controlling the nature of the regenerated appendage. He found that severing the nerve supply to the pincer at the time of snapper amputation prevented transformation of pincer to snapper. Such animals exhibited untransformed regenerated appendages, which were similar to his findings following amputation of both chelae, or following amputation combined with the surgical transection of the nerve supply to both. Perhaps the morphological transformation which occurs following amputation of the snapper is a specific response to transection of motor and/or sensory axons

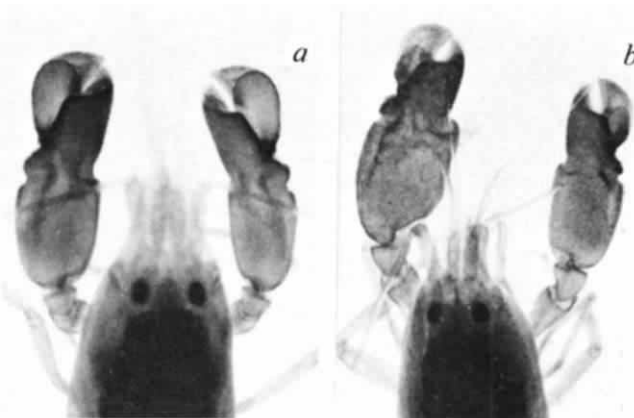
in the leg. To test this we severed both nerves which supply a snapper cheliped, by crushing them just proximal to the coxopodite-basipodite joint. The operation is difficult in that damage to the main artery supplying the leg must be avoided, otherwise necrosis and loss of the entire limb ensue. Furthermore, if the operation is performed more distally, the limb is autotomised within 24 h. Twenty-four control animals were also prepared. They received sham operations in which the arthrodial membrane only was cut.

Table 2 shows the results of these surgical procedures. After eight weeks, all of the living control animals had moulted twice, but none showed signs of limb transformation. Of an original 70 experimental animals, five remained with both claws after two moults, and in all five there was a complete transformation of the pincer claw to a snapper claw without the loss of the original snapper. Photographs of two of these animals are shown in Fig. 2. The original and transformed claws are identical mirror images, and the newly formed snappers were fully capable of functioning as such.

The results of these initial experiments have shown that section of the snapper nerve supply is sufficient to initiate transformation of the opposite claw. The data also suggest that the first control scheme discussed above, in which a message effects transformations of both the opposite and ipsilateral claws following damage to the snapper, is no longer tenable; the damaged snapper is not itself transformed into a pincer.

Whatever the mechanisms of pincer-snapper transformation, our results are unique in their implications for the role of the nervous system in regeneration. We are aware of no previously published report describing morphological and functional transformation of a limb in response to the section of a distant unrelated nervous pathway, apart from data showing that disuse of central nervous pathways during critical periods of development may lead to functional anomalies⁸. It has long been recognised that the central cell bodies of motor neurones, or the target effector organs of these neurones, can undergo functional, histological, and even anatomical changes following section of the associated peripheral axon pathways⁹⁻¹¹. In other cases, the deprivation of sensory input through genetic, surgical or other means can produce changes in the affected target interneurone¹²⁻¹⁴. Each of these effects can be attributed either to

Fig. 2 Individuals of *A. armillatus* (a) and *A. heterochelis* (b) in which the nerve supply to the original snapper claw (left-hand side, each case) had been transected. In both animals, the original pincer claw has been transformed to a snapper.



the direct actions of nerves upon target organs, or to the intracellular consequences of axotomy. In the shrimp, there is no evidence that sensory or motor nerve axons in one claw cross the animal's midline and make functional contact with muscles or other tissues in the opposite claw. Accordingly, even though the details of the anatomical pathways have not yet been determined, the neuronal organisation of crustacean limbs requires that any electrical or humoral information transmitted by nerves from the point of damage to the opposite side must pass across one or more synapse¹⁵. Thus, our results support the interesting possibility that coherent structural changes can be initiated at a distance by the nervous system.

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Loss of phototaxis in silkworm larvae after smelling mulberry leaves and recovery after electroconvulsive shock

NEWLY hatched larvae of the silkworm *Bombyx mori* show a strong phototactic response to ultraviolet (357 nm), green (557 nm) and yellow (585 nm) light, but this is lost rapidly after they are fed on fresh mulberry leaves¹, as reported for some lepidopterous caterpillars^{2,3}. After being fed on an artificial diet containing neither mulberry leaves nor their extract, silkworm larvae continue to be phototactic (our unpublished data). Thus loss of the phototactic response is not caused by the stimulus of feeding, for example through a signal from the pharynx or abdomen. A chemical stimulus from mulberry leaves seems to affect the response directly. Chemicals of plant origin, which are perceived through the sense organs, have been found to elicit or repress some insect behavioural responses⁴, but little is known of the effect of the odour of food plants on the phototactic response of insects. We report here that silkworm larvae temporarily lose their normal phototactic response after smelling mulberry leaves and that a moderate electroconvulsive shock restores the response.

Newly hatched silkworm larvae were laid in a glass test tube inside a large glass box containing fresh mulberry leaves. Thus the larvae were exposed to the odour of the leaves without touching them. Larvae that responded by moving their heads were removed and their phototactic response was measured. Figure 1 shows that the number of larvae showing a phototactic response decreased as exposure to the odour increased, and that the response to all the wavelengths examined (357-700 nm) was lost after 20 min. Larvae transferred from the test tube filled with the odour of mulberry leaves to a clean tube recovered their normal phototactic response in 3 h. After

recovery these larvae repeatedly lost their response if further exposed to the mulberry odour (second and third arrow in Fig. 1). During the second recovery the response was restored faster than in the first. Exposed larvae showed the usual orientation to the light source, and an active chemotactic response to the odour of mulberry leaves.

These observations show that the larvae were not in a state of akinesis or hypnosis caused by the experimental procedures. The possibility that phototaxis might be affected by an odour adhering to the larval integument was excluded because when exposed and unexposed larvae were mixed in the apparatus which measured their responses, only the latter walked to the light source. Furthermore, the threshold of light intensity causing the phototactic response was not increased by exposure to the odour.

Newly moulted larvae of each instar showed the phototactic response, and lost it on exposure to the odour of mulberry leaves as did the hatched larvae. When the antennae (olfactory organs^{5,6}) were removed from newly moulted fifth instar larvae, those larvae did not lose their phototactic response after exposure to the odour. This suggests that the larval antennae perceive the odour that affects the phototactic response.

Some chemicals known to attract silkworm larvae were examined to see whether larvae would lose their response after smelling them. None of the attractants examined—3-hexen-1-ol⁷, citral⁸, terpinylacetate⁸ and dimethyl sulfide⁹—significantly affected the response. Furthermore, the leaves of *Citrus trifoliata*, which attract silkworm larvae, had no effect on the response. These observations suggest that mulberry leaves contain a substance which suppresses the phototactic response of silkworm larvae in addition to other substances, the so called attractants and phagostimulants, which are known to influence larval behaviour^{7-9,10}.

The loss of this response is interesting because, like memory, it continues for some time after the odour stimulus has been removed. Memories produced in the neural system of organisms,

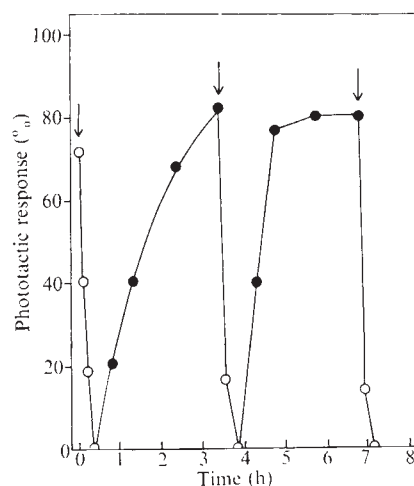


Fig. 1 Loss of phototactic response after smelling mulberry leaves and recovery after transfer of larvae. A slide projector was used to estimate phototactic response. Monochromatic light ($\lambda_{\text{max}} = 585 \text{ nm}$) obtained using an interference filter was projected on to a sheet of white paper fixed inside a black box and the light intensity on the paper was adjusted to $3.7 \times 10^{-2} \text{ W m}^{-2}$. Fifty hatched larvae (3.0 mm long) were placed at a starting area—a stripe 0.5 cm wide—3.0 cm from the white paper. Larvae showing a positive phototactic response orientated themselves rapidly and moved toward the light area spontaneously. The percentage of larvae reaching the paper within 10 min was used as the index of the phototactic response. Newly hatched larvae were placed in a test tube filled with the odour of mulberry leaves, and removed for measurement of the response after 5, 10 and 20 min. Larvae exposed to the odour for 20 min were transferred to a clean, odourless test tube. After recovery of the response, larvae were placed in a test tube filled with the odour (second and third arrow). Exposure to the odour (○) and transfer of the larvae (●) were repeated.

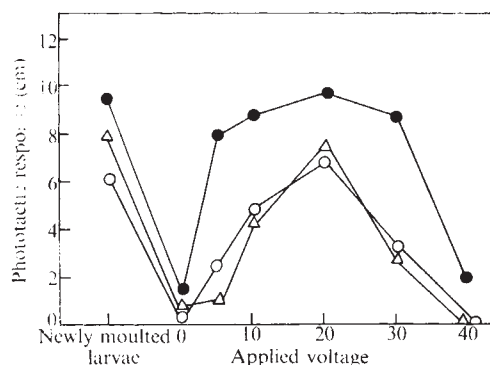


Fig. 2 Recovery of the phototactic response due to electroconvulsive shock. The response was represented by the mean walking distance (cm) from the starting line to the light source. Each point represents an average of three experiments. In one experiment 10 larvae were run for 10 min in an apparatus devised to measure phototaxis. The starting line was 30 cm from the light source. The phototactic response to yellow light was measured and the light intensity at the starting line was adjusted to $2.2 \times 10^{-1} \text{ W m}^{-2}$. Newly moulted third, fourth or fifth instar larvae were exposed to the odour of mulberry leaves for 20 min. The effects of electroconvulsive shock (applied for 2 s) on the response of the exposed larvae were tested for a range of 0–40 V (a.c. 60 Hz). Δ , Phototactic response of third instar larvae; $\circ$, fourth instar; $\bullet$, fifth instar.

including insects, have been reported to be disrupted by electroconvulsive shock after training^{11,12}. Therefore electroconvulsive shock was administered to silkworm larvae after exposure to the odour of mulberry leaves to investigate the role of the neural system in suppressing the phototactic response. First and second instar larvae were too small to be given an electroconvulsive shock, and so newly moulted larvae of the third to fifth instar were used. After exposure to the odour, larvae were placed on aluminium foil which was attached to an electrode and were given a shock by touching the other electrode to the thoracic segment. Twenty volts was most effective for recovering the response of third and fourth instar larvae. With fifth instar larvae, shock was effective in a range of 5–30 V. In all cases more than 30 V had no effect, probably due to paralysis resulting from such a strong shock. When newly moulted larvae of each instar that showed a significant phototactic response were given shocks without pre-exposure to the odour of mulberry leaves, their responses were not affected. Furthermore, those larvae, when given mild shocks, did not walk significantly in the dark, and most of them walked towards the light source when the light was switched on.

The odour of mulberry leaves provides the best available information about the proximity of that food plant to the silkworm larvae, and the loss of the phototactic response might be advantageous in preventing them from moving away. Our findings suggest that nervous impulses resulting from the olfactory stimulus of the odour of mulberry leaves are transmitted to the central nervous system of silkworm larvae, thus controlling phototaxis, and that information which represses phototaxis is stored. The storage of this information may be gradually reduced in the absence of the olfactory stimulus of the odour or it may be extinguished rapidly by electroconvulsive shock. Electrophysiological studies are required to test this hypothesis.

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Is sex determination in germ line and soma controlled by separate genetic mechanisms?

IN sexually dimorphic animals, the determination of sex is a major branch point in development. Although mutations producing sex reversal shed some light on the processes by which this determination is reached, many questions remain unanswered. Autosomal mutations such as *polled* in the goat¹, *sex reversed* (*Sxr*)² in the mouse and *transformer* (*tra*)^{3,7} in *Drosophila* cause chromosomal females to become phenotypic males, although they are sterile and their gonads agametic. The nature of this sterility suggests that the sex of germ cells in *Drosophila*, and perhaps in other species, might be determined by different sets of genes from those which operate in somatic tissue. Here we test this hypothesis by constructing mosaics in *Drosophila* where germ cells of one genotype are surrounded by soma of another. We find that the *transformer* mutation affects only the somatic sexual differentiation, and that it has no effect on the differentiation of germ cells. Thus we conclude that the agametic nature of transformed flies is due to the absence of functional male germ cells and at least with respect to *tra*, germ line and soma have separate sex-determining genes.

In *Drosophila*, the primordial germ cells are derived from a cluster of spherical cells in the perivitelline space outside the cellular blastoderm, while the mesodermal components of the gonad stem from a mid-ventral portion of the cellular blastoderm itself⁹. The separate origin of these two cell types enables one to transplant primordial germ cells between genetically marked embryos, thus making it possible to follow the fate of a germ cell of one genotype when it is surrounded by somatic tissue of another genotype⁴. In *Drosophila*, germ cell transplants yield functional gametes only when the donor and host are of the same sex⁵. Using this sexual restriction of germ-cell function as the basis of our assay, we have transferred primordial germ cells between embryos produced by two genetically marked *transformer* stocks. Our hypothesis predicted that sexual differentiation of germ cells would be unaffected by *tra*, and that, despite the fact that they originated in an animal destined to become a phenotypic male, *X/X;tra/tra* germ cells should produce ova when placed in a female environment. Donor germ cells from *X,y w f/X,y w^a rb f; tra/TM3,Sb Ser* control embryos (that is, normal females) and *X,y w f/X,y^a w rb f; tra/tra* embryos (embryos destined to give rise to transformed flies) were transplanted into *X,y⁺ w⁺ f⁺/X,y⁺ w⁺ f⁺; tra/TM3,Sb Ser* host embryos, which on hatching were mated to *X,y w f/B⁺Y; tra/tra* or *X,y w f/Y; +/+* tester males. The donor stock is marked with the X chromosome markers *y* (yellow body colour); *w* (white eyes); and *f* (forked bristles)⁸, while the host stock is wild type for these markers, so that the progeny which arise from transplanted germ cells can be identified by their *y w f* phenotype (Table 1), and thus flies with mosaic gonads can be readily identified. The genotype of transplanted germ cells with respect to *tra* can be determined by the appearance of the dominant markers *Sb* (short bristles) and *Ser* (serrated wings) among the *y w f* progeny of female hosts test mated with *y w f/Y; +/+* males, and also by the sex of the progeny if the female host was test crossed with *X,y w f/B⁺Y; tra/tra* males (Table 1). Both tester males were used with identical results.

Transplantation of germ cells produced 26 mosaic females. Fifteen of these were classified as having received normal female germ cells (Table 1), as they produced 397 *y w f;Sb Ser* ♀♀ and

Table 1 Phenotypes expected in progeny of female mosaics

Female chromosome segregants*	Tester male ($X,y w f/B^s Y; tra/tra$) chromosome segregants	
	$X,y w f, tra$	$B^s Y; tra$
Host $X; tra$ $X; TM3, Sb Ser$	♂ + ♀ $Sb Ser$	♂ B^s ♂ $B^s Sb Ser$
If donor is female: $X,y w f; tra$ $X,y w f; TM3, Sb Ser$	♂† $y w f$ ♀ $y w f Sb Ser$	♂ $y w f B^s$ ♂ $y w f B^s Sb Ser$
If donor is male: $X,y w f; tra$	♂ $y w f$	♂ $y w f B^s$

*The cross for the donor embryos was $y w f/y w f; tra/TM3 \times y w^a rb f/B^s Y; tra/tra$ (as w^a and rb in combination give white eyes which are indistinguishable from w alone, the two chromosomes $y w f$ and $y w^a rb f$ will not be listed separately here). B^s (Bar stone), which is used to mark the Y chromosome, is a dominant mutation affecting eye shape. $TM3$ is a multiply inverted third chromosome that suppresses crossing over and carries the dominant markers Sb (Stubble) that affects bristles and Ser (Serrate) that affects wing shape. In control counts from the donor stock of over 600 flies, all females exhibited both Sb and Ser , while none of the transformed males expressed either marker, indicating that the penetrance of both these markers is 100% under the conditions used here.

†In some cases $y w f$ tester males with unmarked Y and wild-type third chromosomes were used. In these cases, the genotype of donor embryos is revealed solely by the presence or absence of Sb and Ser progeny in the donor brood whereas, in the example depicted, the sex of the non Bar (B^s) eyed flies is an additional indication of the genotype of the donor germ cells.

$y w f/B^s; Sb Ser$ ♂♂, in addition to 351 $y w f$ flies that did not carry the Sb and Ser markers (Table 2). The presence of progeny carrying Sb and Ser (as well as the presence of female progeny when $y w f/B^s Y; tra/tra$ tester males were used) indicates that the donor genotype was $X,y w f/X,y w^a rb f; tra/TM3, Sb Ser$ (Table 2). Eleven other mosaic females produced exclusively $y w f$ flies without Sb or Ser (594), indicating a donor genotype of $X,y w f/X,y w^a rb f; tra/tra$ (the genotype which produces the transformed phenotype in somatic tissues). Furthermore, nine of these females had been test mated with homozygous tra tester males and their $y w f$ donor progeny were all phenotypic males (that is, 191 transformed flies and 176 B^s males), providing additional evidence for the tra/tra genotype of the donor germ cells.

The rate of mosaic production appears to be the same whether the transplant is tra/tra into female hosts (11/64), normal female into female (15/64), or male into male (18/74) (Table 3). Also, the size of broods obtained from tra/tra germ cells (54 donor progeny/mosaic) is similar to that from normal female germ cells (50 donor progeny/mosaic). Therefore, we conclude that tra does not affect the ability of X/X germ cells to differentiate into ova, nor does it reduce their fecundity. These data demonstrate that $X/X; tra/tra$ germ cells are fully capable of differentiating along the female pathway and that, at least with respect to tra , germ line and somatic tissue do not share a common mechanism of sex determination.

These observations also suggest that the absence of sperm in transformed flies may result from an inability of $X/X; tra/tra$

germ cells to differentiate into sperm. If the agametic nature of transformed flies results from a lack of functional male germ cells, then the transfer of normal male germ cells to transformed flies should lead to the production of sperm in the tra testes and possibly to the restoration of fertility. Judging from the frequency of mosaics in the X/Y male hosts (Table 3, 24%) about 9 of the 38 transformed hosts should have received functional X/Y germ cells. Although none of these 38 transformed males were fertile, dissection of 34 of them revealed that 8 had normal sized testes containing mature non-motile sperm. The frequency of transformed flies with sperm (23%) is almost exactly the same as that expected from the frequency of successful transfer of X/Y germ cells (24%). Thus we conclude that an $X/X; tra/tra$ soma provides an environment which is capable of supporting much of spermiogenesis. This result suggests that the lack of sperm in non-mosaic tra flies (even those of XXY constitution³) is due to the inability of XX germ cells to differentiate into sperm, thereby lending further support to the hypothesis that tra has no influence on the pathway of sexual differentiation that is selected by the germ cells.

Table 3 Results of transplantation of primordial germ cells

Sex of host	Host genotype	Hosts* tested	Fertile mosaics	Donor genotypes
♀ $X; tra$ $X; TM3, Sb Ser$		64	26	15 ♀ $X,y w f; tra$ $X,y w^a rb f; TM3, Sb Ser$ 11 ♂ $X,y w f; tra$ $X,y w^a rb f; tra$
♂ $X; B^s Y; tra$ $B^s Y; tra$ or $tra/TM3$		74	18	10 ♂ $X,y w f; tra$ $B^s Y; TM3, Sb Ser$ 8 ♂ $X,y w f; tra$ $B^s Y; tra$
♂ $X; tra$ $X; tra$		38	0	8 had mature, non-motile sperm in their testes

*Data regarding the number of embryos injected can be found in Table 4. The 74 male hosts and the 38 transformed ♀ hosts were mated to $y w f$ virgins. The 64 surviving female hosts were mated either with $y w f$ males or with $X,y w f/B^s Y; tra/tra$ males. For transfer of pole cells, eggs are collected for 3 h, then dechorionated and immersed in Ringers for selection of blastoderm stage embryos. A donor and host embryo are placed on double stick scotch tape, covered with viscous oil, and the pole cells transferred using a micropipette of 10 µ O.D. Operated embryos were placed in a moist chamber overnight and transferred individually to tubes containing standard fly food supplemented with live yeast on hatching. On eclosion, the appropriate matings were performed.

The tra hosts which had apparently received XY germ cells may be infertile because normal spermiogenesis requires the presence of a Y chromosome in the somatic tissue of the gonad. We therefore transplanted normal X/Y germ cells into $X/0$ male embryos (Table 4). In *Drosophila*, $X/0$ individuals develop as normal males except that they are sterile and the testes contain non-motile sperm. Of 14 $X/0$ males, 5 were made completely fertile by transplantation of normal X/Y germ cells. All of the progeny derived from these mosaic males came from the X/Y donor germ cells. Thus, the action of the Y chromosome is autonomous in the germ line and the infertility of the transformed hosts is not due to the lack

Table 2 Phenotypes of donor progeny

Donor genotype	Mosaic hosts	X/X	X/X^*	X/Y	X/Y	Brood size (donor progeny/mosaic female)
		$y w f Sb Ser$	$y w f$	$y w f B^s Sb Ser$	$y w f B^s$	
$X,y w f; tra$ $X,y w^a rb f; TM3, Sb Ser$	15 ♀ ♀	207	165	190	186	50
$X,y w f; tra$ $X,y w^a rb f; tra$	11 ♀ ♀	0	296	0	298	54

*In some cases the tester males were homozygous for tra , in which cases this class of progeny were phenotypically transformed.

Table 4 Rate of successful transplantation

Embryos injected	Embryos hatching	Adults mated	sterile	fertile	Fertile mosaics
575*	378	176	40§	93	43
50†	39	26	—	15	11

*Cumulative data for all experiments listed in Table 3.

†Cumulative data for experiments involving X/O hosts. Donor embryos were *y w f* and host embryos were from a cross of wild-type virgins with *Y, XY^sB f v Y^L* males (*X^LY*, attached *X/Y* chromosomes). Surviving male hosts were test crossed to *y w f* virgins.

‡Adults mated indicates the number of injected embryos which survived the insults of handling, injection and transfer long enough to be mated and tested for fertility.

§This figure includes the 38 transformed hosts.

of a *Y* chromosome in the somatic tissue, but perhaps to an inability of the transformed gonad to support the final stages of spermiogenesis. The nature and focus of this sterility remains to be established.

Based primarily on observations of adult somatic tissues, Bridges concluded that sex in *Drosophila* is determined by the balance between sets of male-determining genes on the autosomes and female-determining genes on the *X* chromosome⁸. The *tra* locus is autosomal, yet its wild-type function is necessary for normal female development. The occurrence of such autosomal sex reversal mutations, which can apparently override the chromosomal gene balance suggests a possible hierarchy of function involving the chromosomal balance, followed by individual loci which detect or respond to this balance by selecting either the male or female developmental pathways. *tra* and perhaps other single loci affecting sexual differentiation in *Drosophila* as well as other organisms, are best considered as regulatory loci.

Our results demonstrate that *tra* does not influence the determination of sex in germ cells. At the level of organisation exemplified by *tra*, germ line and soma have independent mechanisms of sex determination. In contrast, it has been suggested that imaginal disks may use a combinatorial system of determination^{10–12}, in which the same gene is involved in homologous determinative steps in separate groups of cells of different developmental fate (for example, the decision to become anterior or posterior in the wing disk seems to be controlled by the same gene (*engrailed*) which controls the anterior–posterior decision in the haltere disk). Sex determination in germ line and soma do not seem to be governed by a combinatorial system involving *tra*.

Our observations raise the question of whether germ cells respond to other known autosomal loci producing sex reversal, and if not, to what other loci they do respond. It remains to be established whether dual mechanisms of sex determination are a general occurrence and whether the primary mechanism of sex determination may also be different in soma and germ line.

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Hormone-induced release of intracellular Ca^{2+} triggers meiosis in starfish oocytes

THE oocytes of most vertebrates and invertebrates develop to a particular stage of meiosis and then stop¹. Various external stimuli, such as hormones, sperm or specific proteases² cause them to resume maturation, but it is not known by what mechanism these act. Indirect evidence suggests the involvement of Ca^{2+} in triggering this process in various invertebrate oocytes^{3–6}, and in a few species Ca^{2+} is known to act directly on the oocyte. Such oocytes include those not normally associated with follicular cells, in particular the annelids *Pomatoceros*⁷, *Hydroides*⁵ and *Chaetopterus*⁸ or the lamellibranchs *Barnaea*^{4,6} and *Spisula*⁹. The reinitiation of meiosis under the influence of the divalent cation ionophore A23187 has been demonstrated with oocytes of *Spisula*⁹, *Nereis*¹⁰ and *Chaetopterus*¹¹ or follicle-free *Xenopus* oocytes^{12–13} in the presence of external Ca^{2+} . The assumption that Ca^{2+} can trigger meiosis in starfish^{3,14} has been questioned because it seems that follicle cells release a meiosis-inducing substance when isolated in normal or Ca^{2+} -enriched artificial seawater (ASW)¹⁵, and because the ionophore also activates germinal vesicle-bearing oocytes without inducing nuclear maturation¹⁶. Here we present evidence that Ca^{2+} can act directly on starfish oocytes and that a transient release of intracellular Ca^{2+} is a major step in the action of 1-methyladenine (1-MeAde), the natural hormone which triggers meiosis¹⁷.

Marthasterias glacialis oocytes, collected during May or early June from Roscoff (France) or Oban (Scotland) and prepared free of follicular cells¹⁸ in Ca-free seawater (CFSW), resumed meiosis when the concentration of external Ca^{2+} was increased from 0–10 to 25–300 mM.

We established by the following criteria that this Ca^{2+} -dependent maturation was not caused by the release of 1-MeAde from residual follicular cells. First, the response was as rapid with Ca^{2+} as with 1-MeAde; second, the effect of Ca^{2+} persisted after digestion of the vitelline membrane with Pronase, and third, the response was independent of the assay volume and the number of oocytes used. We found repeatedly that less than 250 follicle-free responsive oocytes resumed meiosis when suspended in 1, 10 or 100 ml of ASW containing 90 mM Ca^{2+} . In addition, biological assays (six experiments) with follicles isolated from 2.5×10^5 oocytes and then incubated in 90 mM Ca-ASW, suggested that all the follicles from 250 oocytes would release no more than 10^{-12} mol of 1-MeAde into the medium. The resulting concentration would still be 10^2 to 10^4 times below the threshold required to reinitiate meiosis.

The possibility that residual follicle cells act by direct contact with the oocyte membrane was excluded because germinal vesicle-bearing oocytes preactivated, by sperm or the ionophore A23187, and which had highly elevated fertilisation membranes, also responded to Ca^{2+} by resuming nuclear maturation. Thus, at least during part of the breeding season, follicle-free oocytes of *M. glacialis* can respond to an increased concentration of external Ca^{2+} . Such Ca^{2+} -stimulated oocytes apparently behave identically to 1-MeAde-stimulated oocytes. For example, they exhibit germinal vesicle breakdown in about 18 min, expel both polar bodies and, when fertilised, develop normally into bipinnaria larvae.

Ca^{2+} - and 1-MeAde-dependent maturation was inhibited competitively by millimolar concentrations of theophylline¹⁹ (Sigma), D600 and isoptin (both Knoll), procaine hydrochloride

Table 1 Percentage inhibition of meiosis reinitiation in seawater containing Ca^{2+} or 1-MeAde

Theophylline	5 mM	85–100%	LaCl_3	10 mM	95–0%
D600	0.4 mM	100–100%	MnCl_2	10 mM	45–100%
Isoptin	0.6 mM	100–100%	Procaine hydrochloride	2 mM	100–100%

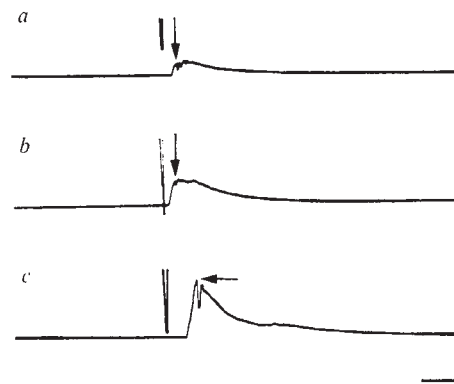
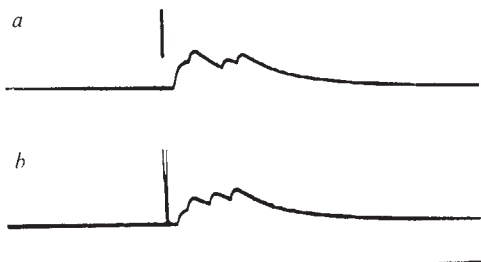
Concentration of Ca^{2+} was 150 mM and of 1-MeAde, 2×10^{-7} M. Counts were made on 300 oocytes, pH adjusted to 8 with Tris-HCl.

(Merck) and Mn^{2+} (Table 1). In addition, 1-MeAde-dependent maturation readily occurred and was even facilitated when the hormone was added after 1 h of preincubation in seawater free of Ca^{2+} and Mg^{2+} and containing either 2 mM EDTA or 2 mM EGTA or in the presence of 10 mM lanthanum chloride. La^{3+} , however, blocks Ca^{2+} -dependent maturation. This raised the question of whether 1-MeAde could act through the release of internal Ca^{2+} .

To investigate this question 120 pl (6 % oocyte volume) of a solution of the photoprotein aequorin²⁰, either from Sigma (type III) or prepared from an extract of *Aequorea fôrskalea* by chromatography²⁰ (dissolved in 10 μM EDTA, 10 mM sodium acetate, pH 6.2), was pressure-injected into oocytes through micropipettes with 0.5–2- μm bevelled tips. Responses were detected without a light guide, using an EMI 9635 photomultiplier displayed on a Tektronix storage oscilloscope. Figure 1 shows the light responses of about 15 injected oocytes after application of external 1-MeAde or Ca^{2+} . Light emission from aequorin was triggered in less than 2 s, before any detectable change in membrane potential. Identical results were obtained with oocytes deprived of their vitelline membrane after treatment with Pronase. Conversely when 1-MeAde was added to a suspension containing 10^6 oocytes per ml of Chelex 100-treated CFSW containing aequorin, no 'flash' was recorded. This indicates that Ca^{2+} is liberated only inside the oocyte.

The peak threshold concentration of Ca^{2+} needed to trigger meiosis, as determined on single aequorin-injected oocytes, was produced by 2×10^{-7} M 1-MeAde and corresponded to a transient change in internal Ca^{2+} concentration of 0.5–1.5 μM . Calibration was achieved as described in ref. 21. The peak light produced by 2×10^{-7} M 1-MeAde was about 20 times less than the value recorded after ionophore activation of an isolated oocyte, which, however, resulted only in the elevation of the fertilisation membrane.

Figure 2 illustrates similar experiments with single aequorin-injected oocytes stimulated with 1-MeAde. These results show that the peak of the free Ca^{2+} change is essential for reinitiation of meiosis because an intracellular injection of EGTA (Na_2 EGTA, pH 6.0) which stopped (Fig. 2a) or inhibited peak light development (Fig. 2b) simultaneously blocked the response.

Fig. 1 Light responses recorded after external application of 10^{-6} M 1-MeAde (a) or 75 mM- Ca^{2+} (b) in CFSW. Oocytes were 'stuck down' by the protamine method²⁷ and their position was noted. In each case, a flash was recorded from about 15 aequorin-injected unsynchronised oocytes which subsequently resumed meiosis. Vertical bar, 0.5 nA; horizontal bar, 10 s. These are original records.**Fig. 2** EGTA inhibition of the response of a single aequorin-injected oocyte to 10^{-6} M 1-MeAde. EGTA (100 pl, 2 mM) was pressure-injected (arrow) either before (a), during (b) or after (c) the peak free Ca^{2+} development. Meiosis occurred only in (c) because in both (a) and (b) the peak Ca^{2+} values remained below the threshold needed for reinitiation. Vertical bar, 0.05 nA; horizontal bar, 10 s. These are original records.

If EGTA was injected after peak light emission (Fig. 2c), meiosis was reinitiated normally. The fact that D600, Mn^{2+} , theophylline and procaine hydrochloride also inhibited competitively both the peak aequorin light emission and the maturation response supports the view that these agents are able to modify the intracellular concentration of Ca^{2+} in starfish oocytes, and this in turn controls reinitiation of meiosis. As far as we know, this is the first direct demonstration *in vivo* of a hormone known to act at the plasma membrane level^{18,22}, triggering a relatively rapid release of intracellular Ca^{2+} which then reactivates cell metabolism^{23,24}. A change in intracellular Ca^{2+} has also been demonstrated during fertilisation of both sea urchin eggs²⁷ and those of the fish *Oryzias latipes*²⁸, as well as being implicated in the reinitiation of meiosis in amphibian oocytes^{25,26}. All these observations suggest that a transient change in intracellular Ca^{2+} within the germ cell constitutes an important and general feature of its future development. This investigation does not explain, however, why the ionophore A23187 can initiate only exocytosis of cortical granule and not nuclear maturation, although it is more efficient than 1-MeAde at releasing intracellular Ca^{2+} . The fact that both of these cellular responses can be triggered independently and successively may indicate either that there are different sites for Ca^{2+} release, or that there is a difference in the responsiveness or localisation of those sites to these agents.

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Direct measurement of intracellular pH during metabolic derepression of the sea urchin egg

SEA URCHIN eggs are shed in a metabolically repressed state and are derepressed by fertilisation. This derepression occurs in two phases, the first of which is caused by an intracellular release of Ca^{2+} (refs 1,2). It has been postulated that the second phase of derepression is accomplished by an increase of intracellular pH^{3-5} . Furthermore, attempts to measure intracellular pH by the use of cell homogenates have supported the idea that intracellular pH is increased during egg activation^{5,6}. We report here direct measurements of intracellular pH of sea urchin eggs during fertilisation and activation by ammonia. These measurements demonstrate the postulated increase of intracellular pH resulting from fertilisation or exposure to NH_4Cl , although the changes in pH differ from those reported in homogenates and seem to be regulated in a more complex manner than originally anticipated.

We measured intracellular pH by means of Thomas type pH sensitive microelectrodes with recessed tips⁷: for our experiments the tip diameter of the outer insulating glass was about 0.5 μm and the distance to the inner pH sensitive surface was 5 μm or less. The pH microelectrodes used gave a linear response of slope 56–59 mV per pH unit within the range pH 2–9 and had less than 3-mV drift when calibrated before and after each experiment in 100 mM phosphate buffers (pH 6.6 and 7.6). The full response times of these microelectrodes were 30 s or less and were unaffected by the presence of high protein concentrations (bovine serum albumin, 50 mg ml⁻¹). Each egg was penetrated by two microelectrodes. Egg membrane potential was measured by means of conventional microelectrodes⁸. The pH sensitive microelectrode, after penetration and healing of the cell membrane, recorded the membrane potential and a voltage proportional to pH. Because of the high resistance of the pH microelectrode, its voltage was fed into a 'hi-impedance' (> $10^{15} \Omega$) amplifier (model F-23, W-P Instruments, New Haven). Both potentials were displayed continuously on separate channels of a Gould-Brush Mark 220 chart recorder. To obtain the intracellular pH, the membrane potential was subtracted from the pH microelectrode record.

Unfertilised eggs of *Lytechinus pictus* were placed in natural seawater (pH 8.1), penetrated with both microelectrodes, and inseminated by the addition of dilute suspensions of sperm. Unfertilised eggs maintained a steady intracellular pH of 6.84 ± 0.02 ($n = 44$). Figure 1 shows a typical response to fertilisation. The rapid increase in intracellular pH closely paralleled the kinetics of the H^+ efflux recorded with extracellular electrodes in the same conditions. Within 5–6 min the intracellular pH of fertilised eggs stabilised at 7.26 ± 0.06 ($n = 8$) and remained close to those values for the 30 min during which we recorded them. These results differ in several respects from pH measurements made on egg homogenates. We did not observe the initial acidification reported before⁹. Our values for the fertilised state show a greater difference from the unfertilised state, and throughout for both unfertilised and fertilised eggs the pH values were higher by 0.3–0.5 units^{5,6}.

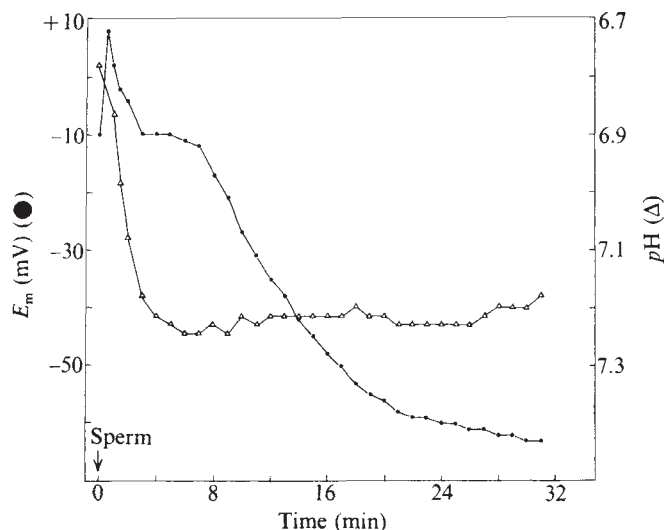
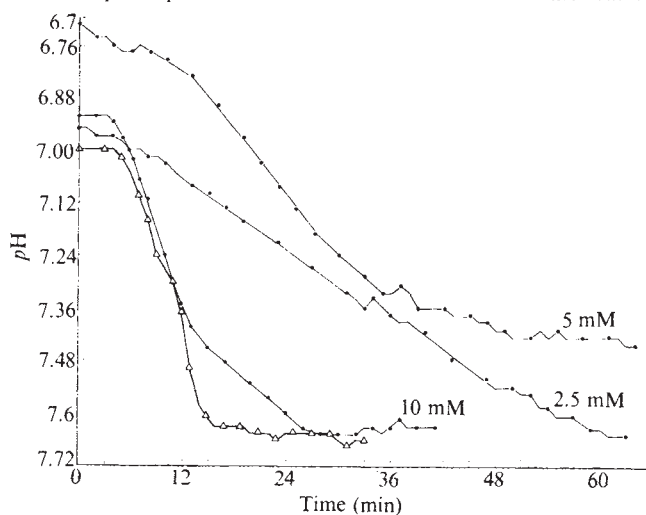


Fig. 1 Continuous recording of membrane potential (E_m) (●) and intracellular pH (Δ) during fertilisation. The conventional microelectrode was inserted into the egg followed by the pH sensitive microelectrode. To ensure minimal damage to the membrane, a current pulse was passed through the conventional microelectrode periodically and the corresponding membrane potential deflection was monitored by the pH sensitive microelectrode. The pH of natural seawater was adjusted to 8.1 with 1 M NaOH in all experiments. Temperature was 17–19 °C in all experiments.

We did not observe a rapid return to low or lower intracellular pH (within 10–20 min) as shown by the most recent measurements⁶ on homogenates.

Numerous artefacts are possible with pH measurements of homogenates. Apart from the obvious difficulties created by the necessary time gap between homogenisation and measurement of pH, it is well established that injury to cell contents releases acid and gives low pH readings⁹. Most of the differences could easily be explained by some degree of cell injury, especially because similar readings can be obtained even after treatment of homogenates with the detergent Triton X-100⁶. The return to low or lower pH values in homogenates may indicate a greater degree of susceptibility to acid release from the cell contents of rapidly metabolising cells when injured. Because the results obtained with homogenates differ widely among themselves and are subject to artefact, we shall question the validity of such results until they are confirmed by more direct measurements on intact cells.

Fig. 2 Continuous recording of intracellular pH during activation by NH_4Cl at 2.5, 5 and 10 mM (Δ) and 5 mM procaine (●). Twice the experimental concentration of NH_4Cl or procaine was dissolved in seawater and the pH was adjusted to 8.1 with 1 M NaOH before the experiment. At $t = 0$, an equal volume of the NH_4Cl or procaine solution was added to the seawater bath.



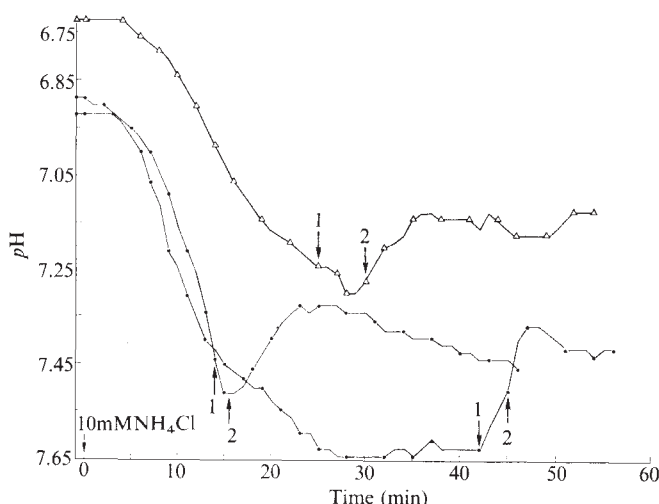


Fig. 3 Continuous recording of intracellular pH during activation by 10 mM NH_4Cl followed by 50-fold dilution (●) or 500-fold dilution (Δ) with seawater. Arrow 1 marks the beginning and arrow 2 marks the completion of dilution.

Using the microelectrode method, we also examined changes in intracellular pH during activation by ammonia. Unfertilised eggs of *L. pictus* were exposed to different concentrations of NH_4Cl and in some cases to procaine (Fig. 2). With the higher concentrations of NH_4Cl , intracellular pH increased more rapidly, as expected from the kinetics of H^+ generation (Winkler, M. M. & Grainger, J. L., in preparation). However, there were two unexpected features. First, H^+ generation stopped well before the change in intracellular pH. Second, given enough time, different NH_4Cl concentrations caused the same increase in pH (0.72 ± 0.01 , $n = 8$) before levelling off. Apparently the intact egg can regulate its intracellular pH and responds only slowly to penetration by a weak base. The pH (7.65 ± 0.05 , $n = 8$) reached in NH_4Cl or procaine was higher than that attained in fertilised eggs and may represent a point at which the intracellular buffers are very effective in resisting further pH increases.

Further evidence of more complex regulation of intracellular pH was seen when the NH_4Cl solution was washed out (Fig. 3). With a 50-fold dilution of a 10-mM NH_4Cl stimulus, there was only partial recovery of lower pH values and then the pH continued to drift slowly upward. With a 500-fold dilution of the 10-mM NH_4Cl stimulus, the intracellular pH again only partially recovered and then remained steady at a level well above the control values for unstimulated eggs.

We conclude that both fertilisation and activation by ammonia result in an increase in intracellular pH throughout the cytoplasm of sea urchin eggs. This increase in pH is a leading candidate in further investigations of the mechanisms of derepression of protein and DNA syntheses in these eggs. Our pH records closely parallel the H^+ efflux during fertilisation, and directly support the proposal⁵ that H^+ efflux is necessary for development because it causes an increase in intracellular pH. The responses to NH_4Cl , involving an increase of intracellular pH, confirm the importance of such an increase for derepression, and they also provide evidence of unknown mechanisms for the regulation of intracellular pH in these eggs.

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Intracellular migration of nuclear proteins in *Xenopus* oocytes

THE protein composition of the amphibian oocyte nucleus (germinal vesicle, or GV) is very different from that of the cytoplasm, as shown by SDS electrophoresis^{1–3}. The oocyte nucleus is known to accumulate components such as RNA polymerase⁴, histones⁵ and factors that protect DNA from nucleases⁶, probably stockpiled for use during early development. Furthermore, some nuclear proteins accumulate in the germinal vesicle after microinjection into the oocyte cytoplasm, as shown by Gurdon⁷ for labelled histones and by Bonner⁸, who injected labelled total GV contents into oocytes. We describe here a study of these phenomena using a recently described high resolution method of protein analysis involving two-dimensional gel electrophoresis⁹. Having defined those proteins which are nuclear, cytoplasmic, or present in both cell compartments of *Xenopus laevis* oocytes, we microinjected a soluble preparation of labelled GV proteins into oocyte cytoplasm and analysed their distribution in the nuclear and cytoplasmic compartments. The results show that cells have exquisitely selective mechanisms that determine the extent to which a protein will become concentrated in the nucleus. Our findings suggest that nuclear proteins contain in their molecular structure a signal that enables them to accumulate in the nucleus. This signal is absent in proteins such as actin, which are similarly abundant in both nucleus and cytoplasm.

Figure 1a–c shows the two-dimensional gel distribution of radioactive proteins in intact *Xenopus* oocytes and in the nuclear and cytoplasmic fractions. By comparing gels 1b and 1c three classes of proteins can be distinguished:

(1) Proteins present both in nucleus and cytoplasm (Fig. 1d). Actin is the most conspicuous member of this class. (In fact, actin is the most abundant nuclear protein when two-dimensional gels are stained with Coomassie brilliant blue. We suggest that actin polymerisation could be responsible for the 'gelation' of the germinal vesicle observed after its isolation in medium containing divalent cations¹⁰).

(2) Nuclear proteins (N proteins), which are highly concentrated in the nuclear fraction; the most abundant of these proteins are designated N1 to N4 (Fig. 1e). Some are extremely acid ($pI \leq 4.5$, see N1, N2 and N4 in Fig. 1e), and are among the most acidic proteins in cells. Although the function of these proteins is unknown, their abundance (shown both by labelling and Coomassie blue staining) suggests a structural function (for example, some of the more acidic proteins could be involved in binding histones, which are known to accumulate in oocyte nuclei in vast excess to the DNA content⁶).

(3) Cytoplasmic proteins which are present in the cytoplasm and not detectable in the nucleus. (Therefore, our manually isolated nuclei must have very little cytoplasmic contamination). The most conspicuous of these proteins is tubulin (Fig. 1f).

We then examined the question of whether nuclear proteins are able to re-enter their normal cellular compartment after microinjection into oocyte cytoplasm. A highly labelled (³⁵S-methionine) preparation of soluble GV proteins was prepared as described in legend to Fig. 2. These proteins presumably came from the nucleoplasm, since the lampbrush

Fig. 1 Fluorographs of two-dimensional gels of ^{14}C -proteins synthesised by *Xenopus* oocytes: *a*, intact oocytes (follicle cells manually removed); *b*, manually isolated germinal vesicles; *c*, manually isolated cytoplasm. *e-f* Show tracings of the three different classes that can be distinguished by comparing gels (*b*) and (*c*). *d*, Proteins present both in nucleus and cytoplasm; *e*, 'nuclear' or N proteins, which are highly concentrated in the nuclear fraction and *f*, 'cytoplasmic' proteins present in the cytoplasm but not detectable in the nucleus. Oocytes were labelled for 24 h in a ^{14}C -amino acid mixture¹⁴ and dissected into GV and cytoplasm after manual removal of the follicle cells. Proteins were extracted, electrophoresed and fluorographed as described previously¹⁴. Very similar results were obtained from oocytes of two different frogs. When proteins are labelled with ^{35}S -methionine (Fig. 3) rather than with ^{14}C -amino acids the relative intensities of the nuclear proteins are somewhat different, reflecting varying amino acid composition. Actin and tubulin were identified as described in ref. 15.

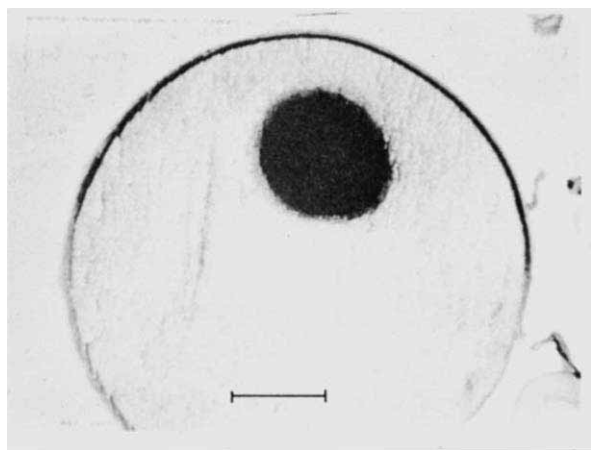
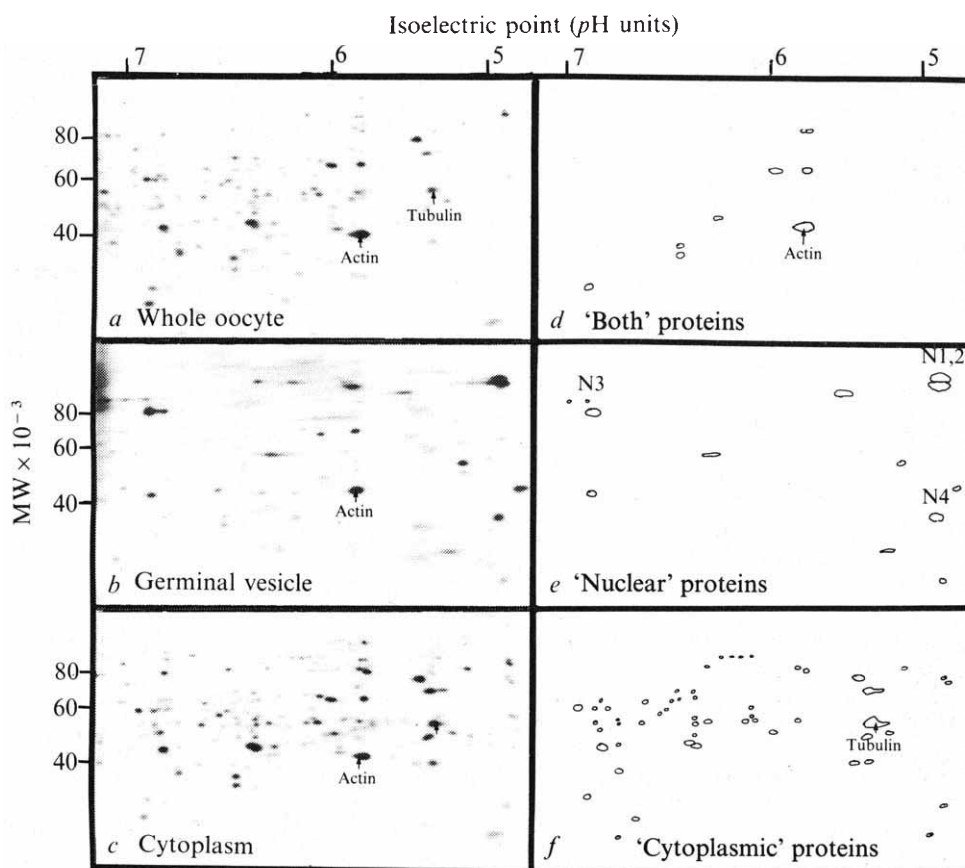


Fig. 2 Autoradiograph of a sectioned *Xenopus laevis* oocyte the cytoplasm of which had been injected 24 h previously with a soluble preparation of ^{35}S -methionine-labelled GV proteins. Injection was aimed at the vegetal pole. The ^{35}S GV proteins were prepared as follows: full-sized oocytes were labelled in 2 μl per oocyte of saline solution containing 2.5 mCi ml^{-1} ^{35}S -methionine (800 Ci mmol^{-1} , Amersham). Oocytes were incubated at 19 °C for 24 h, and cultured in unlabelled medium for an additional 24 h. The GVs (100,000 c.p.m. per GV) were isolated manually and collected in ice-cold isolation medium² containing 10 mM Tris-HCl (pH 7.5), 100 mM KCl, 20 mM NaCl, 3 mM MgCl_2 , 2% w/v polyvinylpyrrolidone (PVP) of 400,000 average molecular weight. PVP preserves the GV structure²; only 1.3% of the trichloroacetic acid-insoluble counts were lost into the isolation medium. The soluble GV proteins were extracted in 1 μl per GV of 10 mM Tris-HCl (pH 8.0) 100 mM KCl, 20 mM NaCl, 3 mM MgCl_2 (without PVP); the GVs were dispersed by sucking up and down in a pasteur pipette, allowed to stand for 1 h at 4 °C, and centrifuged for 1 h at 1,500g. The GV pellet contains the lampbrush chromosomes, nucleoli and nuclear membrane². The soluble supernatant had 91% of the total radioactivity (in agreement with Merriam and Hill who estimated that 97% of the total GV proteins are nucleoplasmic²). Scale bar, 200 μm .

chromosomes, nucleoli and nuclear membrane are pelleted. The labelled proteins were then injected into the cytoplasm of unlabelled oocytes which were cultured for 24 h, and then fixed, sectioned and autoradiographed, or separated manually into nucleus and cytoplasm and analysed on two-dimensional gels. Autoradiography (Fig. 2) showed that most of the radioactivity was distributed uniformly in the nucleus (indicating that most of the labelled proteins are free in the nucleoplasm and not bound to chromosomes or nucleoli); only a small number of grains was found in the cytoplasm.

Figure 3 shows the result of the two-dimensional gel analysis of this experiment. The preparation of labelled GV proteins (Fig. 3b) used for the injections contained actin and all the major nuclear proteins (N1 to N4) found in intact GVs (Fig. 3a). Figure 3c shows the radioactive proteins contained in the GVs reisolated 24 h after microinjection. The major nuclear proteins (N1 to N4) re-entered the nucleus, while the amount of radioactive actin in the nucleus decreased considerably (relative to the N proteins, see Fig. 3c). Conversely, when the cytoplasm of the same oocytes was analysed (Fig. 3d), actin was the most prominent labelled protein, while N1, 2, 3 and 4 were not detectable.

From the results shown in Fig. 3 we can conclude the following: (1) *Xenopus laevis* oocytes contain nuclear proteins which accumulate in the nucleus after microinjection into the cytoplasm, as shown initially by Bonner⁸ and fully confirmed here. (2) The proteins that concentrate selectively in the nucleus after microinjection are located in the nucleoplasm. (3) Nucleus-specific proteins are greatly concentrated in the nucleus, while microinjected actin is found both in the cytoplasm and the GV. This is as expected, since actin is normally present in both cell compartments. Microinjected N1 and N2 were found to be at least 120 times more concentrated in the nucleus than in the cytoplasm. (This was determined by densitometry of the gels shown in Fig. 3c and 1d; readings were corrected so that the intensity of the actin spot was the same in both samples.) (4) The ability of N proteins to enter the nucleus does not depend on the overall charge or size of the molecules. Acidic

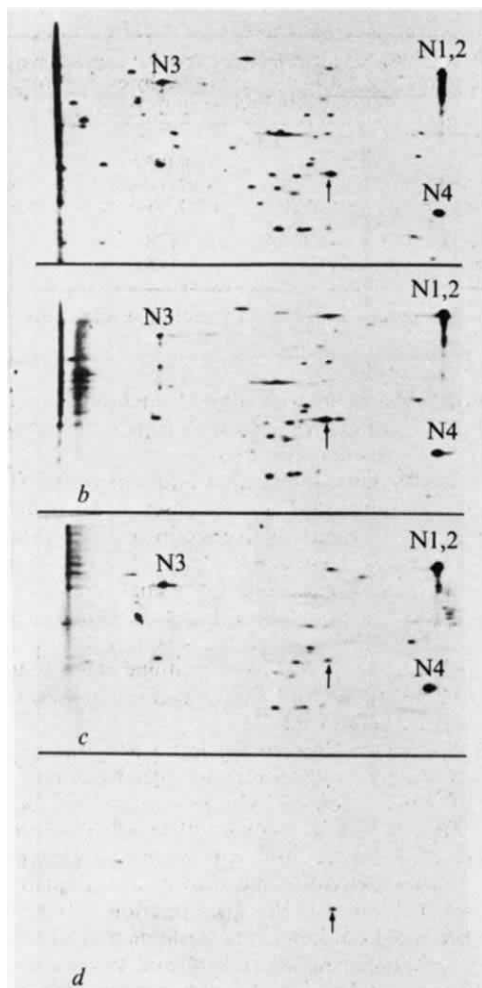


Fig. 3 Two-dimensional gel analysis of microinjected ^{35}S -GV proteins. A soluble preparation of ^{35}S -methionine-GV proteins was obtained as described in Fig. 2 and injected into the cytoplasm of *Xenopus* oocytes. Each injected oocyte received an amount of ^{35}S -protein equivalent to 1/20 of a GV. After injection, the oocytes were cultured for 24 h at 19 °C in unlabelled medium and then dissected into nucleus and cytoplasm. The samples were electrophoresed and fluorographed as described in Fig. 1. The proteins indicated as N1, 2 and 3 in gels (a) and (b) have streaked in the second dimension (this artefact, commonly observed in two-dimensional gels, is due to overheating during the SDS dimension). The vertical arrow indicates the position of actin. a, Total proteins contained in the nucleus of the ^{35}S -methionine labelled oocytes used to prepare the soluble GV proteins. b, Soluble ^{35}S -GV proteins used for microinjection. c, Nuclear fraction from oocytes microinjected with ^{35}S -proteins. d, Cytoplasmic fraction from oocytes microinjected with ^{35}S -proteins.

(N1, 2 and N4), neutral (N3), or basic proteins (histones, see ref. 7) are all concentrated in the GV after microinjection. Similarly, large polypeptides such as N1 and 2 (molecular weight 120,000 and 110,000) and smaller ones such as N4 (MW 35,000) are concentrated with equal efficiency.

Results of earlier studies involving the microinjection of dextrans¹¹, colloidal gold¹² and bovine serum albumin^{7,13} indicate that the oocyte nuclear membrane has functional pores of a radius of about 45 Å (ref. 11). Bovine serum albumin (MW 68,000) enters nuclei only very slowly^{7,13}. However, as shown here, N1 (MW 120,000) enters readily. Furthermore, it is conceivable that *in vivo* N1 could be only a subunit of a still larger molecule. It therefore seems possible that when proteins are nucleus-specific, factors other than size might influence nuclear envelope permeability.

The experiments described here do not test whether concentration within the nucleus is a function primarily of the nuclear membrane or of selective binding to intranuclear molecules that do not traverse the membrane easily.

Our results indicate that nuclear proteins may contain in their molecular structure a signal that enables them to accumulate selectively in the nuclear compartment after microinjection. This signal would be absent in those proteins (such as actin) which do not accumulate preferentially in the nucleus. The precise chemical nature of this signal remains to be determined.

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Two distinct developmentally regulated lectins in chick embryo muscle

EXTRACTS of embryonic chick muscle contain a carbohydrate-binding protein assayed as an agglutinin of treated erythrocytes^{1–4}. Because agglutination is blocked by specific saccharides, the agglutinin is referred to as a lectin by analogy with similar proteins from other sources⁵. Lectin activity changes strikingly with muscle differentiation^{3,4} and is detectable on the surface of muscle cells⁶, suggesting a role in developmentally regulated cell interactions. The lectin has been purified to homogeneity by affinity chromatography⁶. Lactose is a potent inhibitor of this lectin (50% inhibition at 0.3 mM) whereas *N*-acetyl-D-galactosamine is not (no inhibition at 150 mM). We now report a second distinct lectin activity discovered during studies with developing chick muscle. This activity is not detectable with the test erythrocytes used in previous studies, but is readily detectable if the erythrocytes are kept in the refrigerator for 2–3 months. The lectin activity identified by these altered erythrocytes is inhibited by *N*-acetyl-D-galactosamine but not by lactose. Like the lactose-sensitive lectin activity, it changes with muscle development.

To obtain the agglutinins, chick embryos were incubated and pectoral muscles were dissected and homogenised as described by Nowak *et al.*³. Homogenates were made in 20 volumes of 0.15 M NaCl, 2 mM EDTA, 2 mM dithiothreitol, all titrated to pH 9.1 (referred to as DES) with or without added sugars as indicated below. Homogenates were frozen in liquid nitrogen for 5 min, thawed and sedimented at 100,000g for 1 h. The supernatants were dialysed overnight against DES before assay of agglutination activity.

Rabbit erythrocytes were collected in Alsever's medium, washed, treated for 1 h with trypsin (Difco) and fixed with glutaraldehyde and stored, all as described before³. Cells treated in this manner and then kept for up to 1 month in a refrigerator at 6°–8°C are referred to as type 1 erythrocytes; cells kept for 2–3 months in the same conditions are referred to as type 2 erythrocytes. The latter were highly reactive with the newly discovered lectin. Attempts to produce cells with this reactivity by varying the trypsinisation or glutaraldehyde fixation conditions, without ageing, did not give reliable results. However, most preparations of type 1 cells became type 2 cells when refrigerated for 2–3 months. The mechanism of this change is unknown.

Extracts of embryonic chick pectoral muscle agglutinated type 1 and type 2 rabbit erythrocytes (Fig. 1). Type 2 erythrocytes were agglutinated by much lower concentrations of

extract (Fig. 1). Agglutination activity of the extracts varied with muscle development when measured with either type 1 or type 2 erythrocytes. However, the pattern of variation was not identical in both cases. One notable difference was that agglutination activity for type 1 erythrocytes in extracts from 8-d-old chick embryos was about one-tenth that of extracts from 12-d-old chick embryos, whereas with type 2 erythrocytes the 8- and 12-d extracts differed only by about a factor of two. Although other differences are suggested in Fig. 1, the precise time course of changes in lectin activities during development varied somewhat from experiment to experiment. This was due at least in part to the inherent variance in the assay based on serial twofold dilutions.

The difference between the lectin activities detected by type 1 and type 2 erythrocytes was revealed by the effects of 50 mM *N*-acetyl-D-galactosamine or lactose on agglutination due to an extract of pectoral muscle from 16-d chick embryos. With type 1 erythrocytes about 98% of agglutination activity was blocked by 50 mM lactose, whereas the same concentration of *N*-acetyl-D-galactosamine had no detectable effect (Table 1). In contrast, with type 2 erythrocytes 50 mM *N*-acetyl-D-galactosamine inhibited 88% of agglutination activity, whereas 50 mM lactose had no detectable effect (Table 1).

The effect of a series of saccharides on haemagglutination activity of chick pectoral muscle extracts with type 2 erythrocytes is shown in Table 2. *N*-acetyl-D-galactosamine was a potent inhibitor in that it had a detectable effect on agglutination titre at concentrations as low as 0.15 mM (Table 2). However, concentrations of this saccharide up to 15 mM also inhibited no more than one step (50%) of agglutination activity; and only with still higher concentrations was up to 88% inhibition observed. This complex dose-response curve suggests that the lectin activity measured by type 2 erythrocytes is heterogeneous. Thiodigalactoside was also a potent inhibitor, inhibiting haemagglutination by 50% at concentrations of 1.5 mM. This saccharide is also a potent inhibitor of the lectin activity found with type 1 cells, producing 50% inhibition at 0.19 mM with crude extract³ and at 0.15 mM with purified lectin⁶. The differential effects of lactose and *N*-acetyl-D-galactosamine on

Table 1 Effect of *N*-acetyl-D-galactosamine and lactose on agglutination of type 1 and type 2 rabbit erythrocytes by extract from pectoral muscle of 16-d chick embryos

Test erythrocytes	Titre ⁻¹		
	No sugar	50 mM <i>N</i> -acetyl-D-galactosamine	50 mM lactose
Type 1	128	128	2
Type 2	1,024	128	1,024

Agglutination assays and sugar inhibition studies were done as described before³.

lectin activity detectable with type 1 and type 2 cells was confirmed in several experiments with sugars of highest available purity from two manufacturers.

Previous studies have shown that addition of 0.3 M lactose to the homogenising medium increases about tenfold the extraction of the lactose-sensitive lectin assayed with type 1 cells^{3,6}. Therefore we tested the effect of several sugars on the extractability of the lectin activity that could be assayed with type 2 cells. Lactose had little effect (Table 3), further distinguishing the lectin activity assayed with the two cell types. In contrast, *N*-acetyl-D-galactosamine and thiodigalactoside increased about tenfold the extraction of lectin activity assayed by type 2 cells (Table 3).

Another distinction between the lectin activities assayed by type 1 and type 2 erythrocytes was provided by affinity chromatography of chick pectoral muscle extracts on derivatised Sepharose 4B coupled to *p*-amino-phenyl-β-D-lactoside. The column bound all the lectin activity assayed with type 1 erythrocytes, and the activity could then be eluted quantitatively with lactose⁶. In contrast, the agglutination activity assayed with type 2 cells did not bind to the column and was recovered completely with the material that flowed freely through the column. Thus the different lectin activities assayed by type 1 and type 2 cells are not different reactivities of the same lectin acting on different receptors on the two cells; but rather they are discrete molecular entities that can be separated completely by affinity chromatography. However, purification of the *N*-acetyl-D-galactosamine-sensitive lectin has not been achieved.

Fig. 1 Lectin activity of embryonic chick pectoral muscle assayed with type 1 (○) or type 2 (●), trypsinised, glutaraldehyde-fixed rabbit erythrocytes. Extracts were prepared in DES containing both 0.3 M lactose and 0.3 M *N*-acetyl-D-galactosamine and assayed for haemagglutination activity after overnight dialysis against DES. Agglutination assays were done in microtitre V plates, as described before³. Protein was determined by the method of Lowry *et al.*¹¹ with appropriate corrections for the presence of dithiothreitol. Numbers in parentheses indicate the number of independent extracts from each embryonic age that were assayed. The same extracts were assayed with both type 1 and type 2 erythrocytes. The results are mean ± s.e.m.

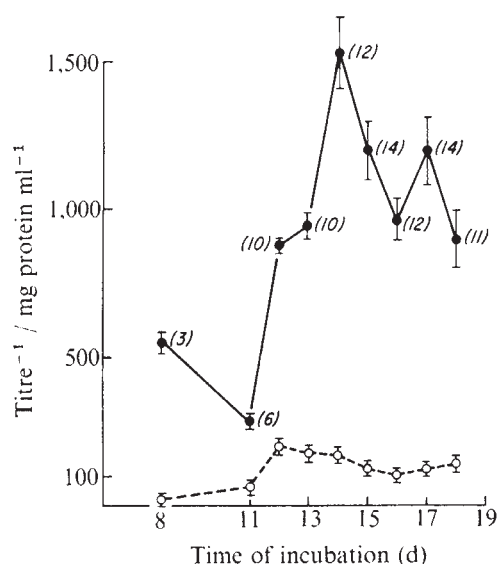


Table 2 Relative potency of saccharides in inhibiting haemagglutination activity of pectoral muscle extracts from 15-d chick embryos

Saccharide	Concentration that inhibits 50% (mM)
<i>N</i> -acetyl-D-galactosamine	0.15
Thiodigalactoside	1.5
Melibiose	37
Methyl-β-D-galactoside	75
D-galactose	75
Methyl-α-D-galactoside	150
Lactose	150
D-mannose	150
L-fucose	150
D-glucose	> 150
<i>N</i> -acetyl-D-glucosamine	> 150
D-fucose	> 150
<i>N</i> -acetyl mannosamine	> 150

Results are the average of determinations made on two separate extracts, using type 2 erythrocytes. The results were obtained by determining inhibition of haemagglutination activity by a range of concentrations of each saccharide. The controls showed titres of 1:1,024. The minimal concentration of saccharide that reduced the titre to 1:512 is taken to be the concentration that inhibits haemagglutination activity by 50%. However, with the lectin activity assayed here, dose-response curves were not simple. For example, *N*-acetyl-D-galactosamine which inhibited haemagglutination activity by 50% at concentrations as low as 0.15 mM inhibited by no more than 50% until concentrations exceeded about 15 mM, and, as shown in Table 1, inhibited by only 87.5% (titre of 1:128) even at concentrations as high as 50 mM. Higher concentrations did not reduce the titre any further. This suggests that the lectin activity that is being assayed is heterogeneous.

Table 3 Effect of addition of saccharides to homogenising medium on extraction of lectin activity

Addition to DES	Titre ⁻¹ /mg protein ml ⁻¹
None	192
0.3 M lactose	309
0.3 M <i>N</i> -acetyl-D-galactosamine	2,510
0.3 M thiodigalactoside	3,048

Aliquots of embryonic chick pectoral muscle were homogenised in DES containing saccharide, as indicated. The extracts were centrifuged at 100,000g for 1 h and the supernatant was dialysed overnight against DES before determination of haemagglutination activity with type 2 erythrocytes.

We attempted this by adsorption of this lectin to type 2 erythrocytes followed by specific elution, a method applied to other lectins⁷. Adsorption was done in the presence of 0.3 M lactose, to prevent concurrent binding of the lactose-sensitive lectin. The *N*-acetyl-galactosamine-sensitive lectin was adsorbed quantitatively in these conditions. Some protein could then be eluted specifically from the cells with *N*-acetyl-galactosamine but only a small fraction of the lectin activity was eluted and it was very unstable. Other purification procedures are being attempted.

Our experiments demonstrate two distinct developmentally regulated lectins in embryonic chick pectoral muscle. Gartner and Podleski⁸ reported that some of the haemagglutination activity they found in muscle was not inhibited by thiodigalactoside, but the mechanism of haemagglutination was not determined. Their haemagglutination activity could not be identified as lectin activity because there was no evidence that it was mediated by interaction with saccharide residues on the test cells. In contrast, the distinct agglutinin activity we have detected with type 2 cells is clearly due to a lectin, as indicated by its inhibition by a specific group of saccharides.

There has been speculation about the possible function of the lactose-sensitive lectin in muscle development. Its possible role in muscle fusion has been considered^{2,4,8}. The demonstration that the lectin is detectable on the surface of muscle cells⁶ supports the possibility that it, like developmentally regulated lectins in slime moulds⁹, mediates specific cellular interactions in differentiation. The demonstration of a second distinct, developmentally regulated lectin activity in embryonic chick muscle is reminiscent of the situation in the slime mould *Dictyostelium discoideum*, which contains two distinct developmentally regulated lectins which have been purified and shown to be products of different genes¹⁰. The significance of two such lectins in differentiating tissue remains to be determined.

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Survival of sucrose-loaded erythrocytes in the circulation

In an attempt to clarify the mechanism of cell lysis under intense electric fields^{1–5}, we have found that aqueous pores are introduced into human erythrocyte membranes when an isotonic suspension of red cells is exposed to an electric field of a few kV cm⁻¹ for a duration in μ s range. These pores are formed when the transmembrane potential induced by the externally applied field exceeds a critical value of 1 V. The effective radius of the pores is several Å, and can be varied by the adjustment of field intensity, field duration, and the ionic strength of the medium. The pores remain open at low temperatures but close completely on incubation at 37 °C. In a proper medium, the resealing of perforated cells takes place without haemolysis, allowing us to prepare erythrocytes (not ghosts) of altered intracellular composition. In particular, foreign molecules such as sucrose have successfully been incorporated into resealed erythrocytes, which were apparently intact at least in terms of cell volume, cell shape, glucose transport, and Na–K pump activity⁴. Thus we have suggested that erythrocytes loaded with a drug by this technique might serve as intravenous drug reservoirs which slowly release the drug molecules into the circulation. Here we demonstrate that erythrocytes loaded with sucrose survive in the circulation with a lifetime almost indistinguishable from that of normal cells, and that the sucrose remains entrapped within the cells. For drugs that slowly permeate the erythrocyte membranes, therefore, our technique offers a means of sustaining a low plasma level for a long period of time, and this could be advantageous in clinical and other situations.

Mice, AKR/J female, were used throughout the experiments. The response of mouse erythrocytes to pulsed electric fields is quite similar to that of human erythrocytes, as is shown in Fig. 1 and Table 1. Figure 1 shows that an electric field greater than

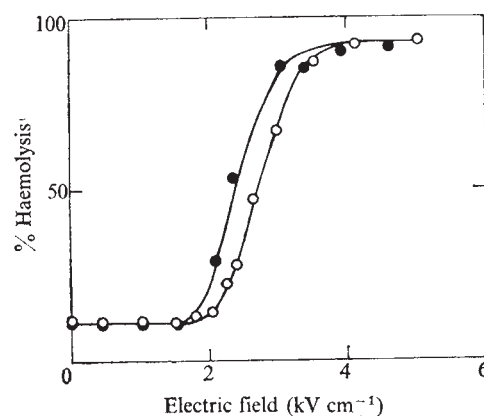


Fig. 1 Haemolysis of mouse erythrocytes as a result of voltage-induced pore formation. ○, 20- μ s pulse; ●, 80- μ s pulse. Blood was collected from eyesockets of AKR/J female mice into heparinised capillary tubes. Erythrocytes were washed by centrifugation at 4 °C twice with a washing medium (NaCl 150 mM, KCl 8 mM, Na-phosphate buffer 6 mM, MgSO₄ 2 mM, D-glucose 10 mM, adenosine 1 mM, inosine 1 mM, penicillin G 500 units ml⁻¹, chloramphenicol 0.1 mg ml⁻¹, pH 7.5) and once with an incubation medium (washing medium plus bovine serum albumin at 3.5% v/v), and finally resuspended in the incubation medium at a volume concentration of 6%. An aliquot of the suspension was warmed to 25 °C and exposed to a single square-wave electric pulse of indicated intensity and duration in a device described elsewhere³. Immediately after the pulse treatment, the suspension was diluted with 12 volumes of ice-cold incubation medium containing 2 mM CaCl₂, and kept at 4 °C with occasional mixing. After 20 h, the sample was centrifuged and the extent of haemolysis determined from the concentration of haemoglobin in the supernatant (cyanmethaemoglobin method⁶). The value corresponding to 100% haemolysis was obtained by hypotonic lysis in water.

about 2.5 kV cm^{-1} induces haemolysis of mouse red cells. The haemolysis is the result of the osmotic imbalance caused by the destruction of the permeability barrier, and is directly correlated with the formation of pores^{3,5}. From the data in Fig. 1 and the dimension of mouse erythrocytes ($5.8 \mu\text{m}$ in diameter, ref. 7, and $49 \mu\text{m}^3$ in volume, ref. 8), the critical transmembrane potential at which pores are formed in mouse erythrocyte membranes was calculated to be 1.0 V for the $20\text{-}\mu\text{s}$ pulse and 0.9 V for the $80\text{-}\mu\text{s}$ pulse, in good agreement with the values for human erythrocytes⁵. The rather high level of haemolysis in untreated cells is because these mouse erythrocytes are more fragile than human erythrocytes. If the incubation medium in Fig. 1 does not contain Ca , Mg and glucose, untreated mouse erythrocytes almost completely haemolyse within 20 h, whereas fresh human erythrocytes can be kept in isotonic NaCl for days.

In the experiment shown in Table 1, erythrocytes were treated with an electric pulse in the presence of [^{14}C]-sucrose. As in the case of human erythrocytes^{4,5}, the size of the voltage-induced pores was found to be strongly dependent on pulse duration, and pores which admit sucrose molecules were obtained when the duration exceeded $20 \mu\text{s}$. Subsequent incubation at 37°C allowed resealing of the leaky erythrocytes, while stachyose was added to the medium to prevent haemolysis¹. Thus, at the end of the incubation, we obtained apparently intact erythrocytes which had entrapped sucrose inside. Again, a considerable degree of haemolysis was observed during the resealing procedure, in contrast to the nearly 100% survival of human erythrocytes through a similar treatment⁴.

The erythrocytes loaded with [^{14}C]-sucrose were injected back into mice from a tail vein. The top curve in Fig. 2 represents the level of sucrose remaining in circulating erythrocytes. The curve starts from 100% recovery, which was calculated from the injected amount and the total erythrocyte volume in a mouse, and declines with a half life of 14 d, or approximately 18 d after correction for the loss by bleeding; this is within the range of the known half life of mouse erythrocytes of 15–20 d (ref. 9). The data show, therefore, that the loaded erythrocytes were essentially intact, and that the erythrocyte membranes had completely regained their impermeability to sucrose. In the second curve, the resealing period was reduced to 1 h, whereupon half of the injected sucrose had disappeared from the circulation by 12 h. This is probably due to lysis of, rather than to leakage of sucrose molecules from, incompletely resealed cells, because *in vitro* experiments on human erythrocytes showed that incubation at 37°C for 1 h is

Table 1 Incorporation of sucrose in mouse erythrocytes

Pulse duration (μs)	0	10	20	40	80	160
Sucrose content (mmol l^{-1} cells)	0.1	0.3	0.5	1.4	3.5	6.9
% cells surviving	98	98	97	96	91	78

Washed erythrocytes were prepared as described in the legend to Fig. 1. The cells were suspended at 20% v/v in a mixture of 76 volumes of the incubation medium, 15 volumes of 270 mM stachyose, and 9 volumes of 290 mM sucrose containing [^{14}C]-sucrose at a final specific activity of $0.4 \text{ mCi mmol}^{-1}$. The suspension was treated with a 3.5 kV cm^{-1} pulse of indicated duration at 25°C , immediately transferred into a plastic tube, and incubated at 37°C in a shaker bath. After 1 h, the cells were centrifuged down, resuspended at 5% v/v in a mixture of 85 volumes of the incubation medium containing 2 mM CaCl_2 and 15 volumes of 270 mM stachyose, and incubated at 37°C for an additional 5 h. Aliquots of the resealed erythrocytes were packed in microhaematocrit tubes, bleached with perchloric acid/hydrogen peroxide, mixed with ascorbic acid and Hydromix (Yorktown Research), and counted in a Beckman liquid scintillation counter. The % of cells which survived the loading procedure was estimated from the amount of haemoglobin released during the two incubations. The procedure described above is a simplified version of the method in ref. 4 (in Table 1 of this ref., Na and K contents in untreated erythrocytes were interchanged by mistake).

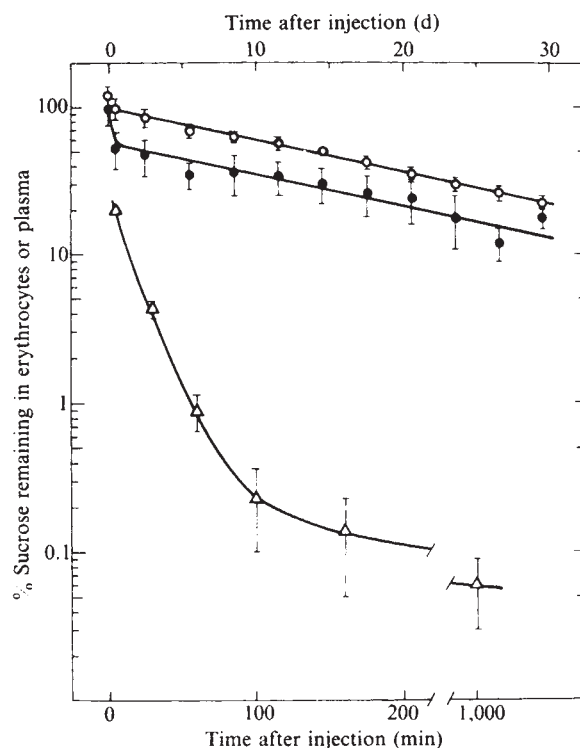


Fig. 2 Elimination of sucrose from circulation. $\circ$, sucrose entrapped in fully resealed erythrocytes; $\bullet$, sucrose entrapped in partially resealed erythrocytes; $\triangle$, free sucrose. In the upper two curves (upper abscissa) erythrocytes were loaded with [^{14}C]-sucrose as described in Table 1, using an $80\text{-}\mu\text{s}$ pulse and incubation periods of $1+5 \text{ h}$ (full resealing) or $0.5+0.5 \text{ h}$ (partial resealing). The loaded erythrocytes were washed twice with the washing medium and resuspended at about 35% v/v in the same medium. Mice weighing 24–30 g were injected with 150–250 μl of the final suspension which contained approximately $0.3 \mu\text{mol}$ or $2 \mu\text{Ci}$ of [^{14}C]-sucrose (in one experiment, a lower specific activity of $0.4 \text{ mCi mmol}^{-1}$ was used, giving the same result). In the bottom curve (lower abscissa) $7 \mu\text{mol}$ or $3 \mu\text{Ci}$ of free [^{14}C]-sucrose in 250 μl of the washing medium was injected into each mouse. At intervals after the injection, approximately 40 μl of blood was withdrawn from an eye socket of each mouse, and radioactivities in erythrocytes and plasma were determined as described in Table 1. Total activities in a mouse were calculated by assuming that 30 μl of erythrocytes and 50 μl of plasma were contained per g body weight¹⁰. Each point is an average of 3–8 mice, the vertical bar being the standard deviation. The mice were apparently in a state of mild shock for a few hours after the injection (haematocrits were abnormally high), and this probably accounts for the slightly over 100% recovery in the top curve at 5 min.

sufficient to reseat the membranes against sucrose, mannitol or xylitol, but not to small ions such as Na^+ or K^+ , which, although very slowly, leak through the membranes (K.K. and T.Y.T., unpublished). The other half of the injected erythrocytes, however, survived in the circulation with the normal half life. When free sucrose was injected into a vein, it quickly disappeared from the plasma, as shown in the bottom curve of Fig. 2. In contrast, the plasma concentration of sucrose in the presence of loaded erythrocytes (the upper two curves) was held at roughly 1/1000 the concentration in erythrocytes for the whole 30-d period.

Many systems, including erythrocytes, liposomes and nylon microcapsules, have been proposed as vehicles of drugs, the aims being prolonged plasma level, possible targeting, or protection against inactivation (for a review, see ref. 11). Of these, erythrocytes seem to be the most attractive because of their natural abundance and long life span (120 d in human¹²) in the circulation. Although past proposals on erythrocytes^{13–16} have almost exclusively focused on the use of ghosts (which are difficult to reseat completely) as vehicles of macromolecules such as enzymes, intact erythrocytes could be used as reservoirs of small-sized drugs, as we have shown above. Many drugs are

known to be rapidly removed from the circulation with a time course more or less similar to that of free sucrose above¹⁷, and so entrapment in erythrocytes could increase the efficiency of the drugs by sustaining the plasma level for a longer period of time. Specifically, (1) frequency of injection could be reduced; (2) for a drug that is toxic at high concentrations, the entrapment assures a low plasma level under a relatively high dosage; inflammation at the site of injection, for example, could be avoided; (3) the relatively constant plasma level might enable accurate targetting if target cells have a somewhat lower threshold than other cells; (4) for a drug that does not permeate erythrocyte membranes, targeting of the reticuloendothelial system is possible. Loading by the present voltage-pulsation technique is applicable to those drugs with a molecular weight of the order of several hundreds (large pores would be difficult to reseal), those which are soluble in water, those which permeate erythrocyte membranes only slowly, and those which do not impair the viability of erythrocytes.

Finally, we point out that the loading technique described here, which could be applicable to cells other than erythrocytes⁵, may be useful not only for medical application, but also for basic researches such as membrane transport and intracellular metabolism.

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Potentialiation of human erythropoiesis *in vitro* by thyroid hormone

ANAEMIA is a frequent complication of thyroid dysfunction in man, and often remains unexplained, responding only to thyroid hormone replacement therapy^{1,2}. Thyroid hormone has recently been shown to influence the rate of red blood cell and haemoglobin production^{3–7}. In addition to stimulating erythropoietin (Ep) production³, thyroid hormone has been reported to act directly on haematopoietic precursors^{4,5}. The enhancement of murine and canine *in vitro* erythroid colony growth by thyroid hormone^{6,7} suggests that this hormone may have a direct role in the modulation of erythroid proliferation. Studies of murine erythropoiesis support the existence of two distinct, age-structured, Ep responsive populations of committed erythroid precursor cells: the early committed erythroid burst

forming unit (BFU-E) and the late committed erythroid colony forming unit (CFU-E)⁸. These populations are distinguished by cell volume, responsiveness to Ep, and proliferative capacity. The BFU-E, a cell which arises from the pluripotent stem cell soon after its commitment to the erythroid line and which has a high Ep-responsive proliferative capacity, seems to be the precursor of the CFU-E, a cell of lower proliferative capacity. Thyroid hormone has been shown to enhance *in vitro* erythroid colony formation by canine marrow cells, an effect that may be mediated by a receptor with β_2 -adrenergic properties⁷. Velocity sedimentation analysis revealed that colony-forming cells stimulated by this hormone sedimented more slowly than colony-forming cells responding to Ep alone⁷. Here, we have extended these studies to human marrow cells and have shown that the ability of thyroid hormone to stimulate the erythroid committed progenitor cell does not seem to be a function of the state of differentiation of this cell. Both CFU-E-derived and BFU-E-derived colony formation were enhanced by the hormone.

We added thyroid hormone to human bone marrow cells cultured in the plasma clot culture system described by Tepperman *et al.*⁹. Table 1 lists the results of CFU-E-derived colony formation obtained after culture for five days. Addition of L-thyroxine (L-T₄) at an optimal final concentration of 10⁻⁸ M and L-triiodothyronine (L-T₃) at 10⁻⁹ M to cultures containing Ep resulted in doubling and tripling, respectively, of the mean number of CFU-E-derived colonies formed (Table 1). On the other hand, the addition of D-thyroxine (D-T₄) and D-triiodothyronine (D-T₃) to cultures containing Ep had no effect (data not shown). Neither L-T₄ nor L-T₃ stimulated the formation of CFU-E-derived colonies in the absence of Ep. When DL-propranolol was added at 10⁻⁸ M final concentration, complete reversal of enhanced colony formation by thyroid hormone was observed. Similar results were obtained in three additional studies. Varying the concentration of DL-propranolol added to the culture mixture revealed that inhibition of enhanced colony formation by thyroid hormone was no longer observed at 10⁻¹⁰ M final concentration of DL-propranolol (data not shown). DL-propranolol added to cultures containing Ep alone had no effect on *in vitro* erythropoiesis (Table 1).

It is noteworthy that L-T₄ and L-T₃ stimulation of CFU-E-derived colony formation was seen over the full range of Ep concentrations (Fig. 1a). Furthermore, when optimal concentrations of L-T₄ and L-T₃ were added to cultures containing varying concentrations of marrow cells and 2.0 IU ml⁻¹ of Ep, CFU-E-derived colony formation was observed to be linearly related to the number of cells plated and to be greater in each instance than colony formation in cultures containing Ep alone (Fig. 1b).

Table 1 Effect of L-T₄, L-T₃ and DL-propranolol on CFU-E-derived erythroid colony formation of normal human bone marrow

Addition to culture	No. of CFU-E-derived colonies per 6 × 10 ⁵ nucleated cells
None	0 ± 0
Ep alone	473 ± 75
Ep + L-T ₄ (10 ⁻⁸ M)	1,018 ± 55 (<i>P</i> < 0.05)
Ep + L-T ₄ (10 ⁻⁸ M) + DL-propranolol (10 ⁻⁸ M)	676 ± 154 (<i>P</i> > 0.10)
Ep + L-T ₃ (10 ⁻⁹ M)	1,331 ± 113 (<i>P</i> < 0.05)
Ep + L-T ₃ (10 ⁻⁹ M) + DL-propranolol (10 ⁻⁸ M)	448 ± 67 (<i>P</i> > 0.10)
Ep + DL-propranolol (10 ⁻⁸ M)	456 ± 55 (<i>P</i> > 0.10)

L-T₄ and L-T₃ (Sigma) were added to the culture medium at the concentrations shown, in the presence of 2 IU per ml of human urinary Ep; in some cases, DL-propranolol (Sigma) was added at a concentration of 10⁻⁸ M. After 5 d, plasma clots were removed from incubation, fixed with glutaraldehyde and stained with dimethoxybenzidine and haematoxylin. The number of CFU-E-derived colonies was determined under ×100 magnification, counting only those colonies composed of eight or more benzidine-positive cells. Values represent the mean ± s.e.m. *P*-values represent comparison to Ep alone, using the two-sample ranks test of Wilcoxon–White.

The baseline number of colonies formed in the presence of Ep alone is different in separate experiments (Table 1 and Fig. 1). This variability is due to the fact that marrow from different normal volunteers was used in different experiments, and it is not unexpected that different individuals have different numbers of committed erythroid stem cells per 6×10^5 nucleated marrow cells at any given time. In addition, the number of colonies obtained after the addition of L-T₃ to the culture medium in some instances was greater than that obtained after supplementation with L-T₄. This was not a consistent observation, however, and the cause of the occasional superior enhanced colony formation by L-T₃ is unknown.

When normal human marrow is cultured in the presence of Ep, BFU-E-derived colonies appear after 12–14 d of incubation. Termination of our cultures after 12 d of incubation allowed us to determine the effect of thyroid hormone on this primitive stem cell (Table 2). Both L-T₄ and L-T₃ enhanced BFU-E-derived colony formation of normal human marrow cells in the presence of Ep, while neither stimulated colony formation in the absence of Ep (Table 2a). Optimal final concentrations required for L-T₄ and L-T₃-induced BFU-E derived colony enhancement were 10^{-9} M and 10^{-11} M, respectively (Table 2a). Similar final concentrations of D-T₄ and D-T₃ had no effect on BFU-E-derived colony formation (data not shown). The stimulatory effect of thyroid hormone on Ep-dependent BFU-E-derived colony formation was reversed by the addition of DL-propranolol to the culture mixture (Table 2b), an effect that was no longer present when the final concentration of DL-propranolol was reduced to 10^{-11} M.

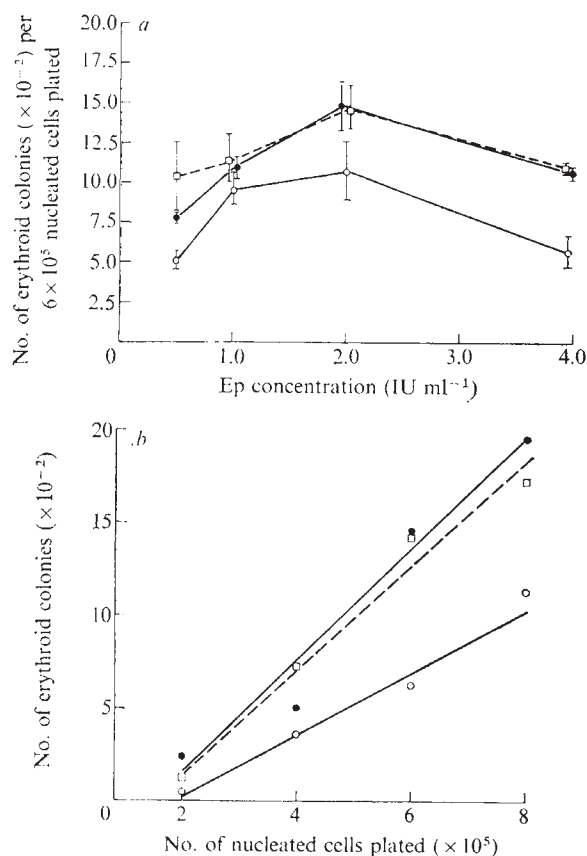
These findings demonstrate that thyroid hormone enhances human red blood cell production by stimulating the proliferation

Table 2 Effect of L-T₄, L-T₃ and DL-propranolol on BFU-E-derived erythroid colony formation of normal human bone marrow

a	Addition to culture	No. of BFU-E derived colonies per 6×10^5 nucleated cells
	None	0
	L-T ₄ (10^{-9} M)	0
	L-T ₃ (10^{-11} M)	0
	Ep alone	182 ± 18
	Ep + L-T ₄ (10^{-8} M)	469 ± 78 ($P < 0.05$)
	Ep + L-T ₄ (10^{-9} M)	545 ± 95 ($P < 0.05$)
	Ep + L-T ₃ (10^{-10} M)	303 ± 87 ($P < 0.05$)
	Ep + L-T ₃ (10^{-11} M)	462 ± 33 ($P < 0.05$)
b		
	Ep alone	228 ± 48
	Ep + L-T ₄ (10^{-8} M)	396 ± 60 ($P < 0.05$)
	Ep + L-T ₄ (10^{-8} M) + DL-propranolol (10^{-8} M)	252 ± 35 ($P > 0.10$)
	Ep + L-T ₄ (10^{-8} M) + DL-propranolol (10^{-11} M)	329 ± 49 ($P < 0.10$)
	Ep + L-T ₃ (10^{-10} M)	388 ± 32 ($P < 0.05$)
	Ep + L-T ₃ (10^{-10} M) + DL-propranolol (10^{-8} M)	280 ± 31 ($P > 0.10$)
	Ep + L-T ₃ (10^{-10} M) + DL-propranolol (10^{-11} M)	366 ± 22 ($P < 0.05$)

Erythroid colonies were cultured in the presence and absence of 2.0 IU per ml Ep for 12 d. Only clusters of three or more aggregates of 8–49 benzidine-positive cells or single clusters of 50 or more benzidine-positive cells were scored as BFU-E derived colonies. Values represent the mean $\pm$ 1 s.e.m. P -values represent comparison to Ep alone (see legend to Table 1).

Fig. 1a The effect of varying concentrations of Ep with and without (○) L-T₄ (10^{-8} M) (●) and L-T₃ (10^{-9} M) (□) on human erythroid colony formation (CFU-E). Similar results were obtained in five additional studies. **b** The effect of various cell concentrations on erythroid colony growth (CFU-E) with L-T₄ (10^{-8} M) (●) and L-T₃ (10^{-9} M) (□) in the presence of 2.0 IU Ep compared to growth with 2.0 IU Ep alone (○). Each value represents the mean of the results obtained.



and/or differentiation of both primitive and more differentiated, erythropoietin-responsive stem cells. This effect is entirely dependent on Ep, the primary physiological regulator of erythropoiesis¹¹. β -Adrenergic agonists enhance canine marrow erythroid colony growth, an effect that may be mediated by surface receptors having β_2 subspecificity¹². The receptor mediating potentiation of human erythropoiesis by thyroid hormone also seems to have β -adrenergic functional characteristics, as enhanced erythropoiesis is reversed by the competitive β -adrenergic inhibitor DL-propranolol, an effect overcome by decreasing the relative concentration of inhibitor present by 100-fold for CFU-E-derived colony formation, and by 1,000-fold for BFU-E-derived colony formation. Such receptors may be sterically altered or increased in number by thyroid hormone^{13,14}, resulting in enhanced haematopoietic stem cell proliferation and/or differentiation in response to β -adrenergic catecholamines present within the plasma clot.

The observation that the optimal concentration for L-T₃ potentiation of erythropoiesis is lower than that for L-T₄ augmentation is consistent with the known physiological potency of these hormones. Furthermore, the optimal concentrations for BFU-E-derived colony enhancement by both L-T₄ and L-T₃ are less than that required for optimal CFU-E-derived colony enhancement, suggesting that the less differentiated stem cell is more sensitive to the effects of thyroid hormone than is the more mature precursor. In contrast to the reported enhancement by D-T₄ and D-T₃ of canine erythropoiesis⁷, these nonphysiological analogues had no effect on human erythroid colony formation in the concentrations tested.

These studies suggest that thyroid hormone may have an important role in normal human erythroid proliferation and/or differentiation. Ep, although the primary regulator of erythropoiesis, appears to require a suitable hormonal milieu in order to function optimally. The abnormalities of erythropoiesis that characterise thyroid dysfunction in man are more easily understood in the light of the potentiating effect that this hormone has on normal human erythropoiesis.

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Isolation method affects transformed cell line karyotype

NEOPLASTIC cells frequently have a karyotype deviant from the normal diploid state¹. Transformation of cells in culture by tumour viruses can be used as a model of neoplastic transformation^{2,3} and is generally considered to be accompanied by extensive changes in chromosome number and morphology⁴⁻⁷. It has not been clear whether these changes are the cause or the result of transformation. It has been suggested that for one DNA tumour virus, simian virus 40 (SV40), the initial step in transformation is the formation of polyploid cells^{5,8,9} and near-diploid cells in some SV40-transformed cell lines are thought to arise from multipolar mitoses⁹. In contrast, stable diploid, or occasionally pseudodiploid transformed cell lines have been obtained after transformation by various tumour viruses¹⁰⁻¹⁷. Explanations for these apparently contrasting results could be based on differences in the origin of the transformed material¹⁸ or on the method of transformation. We have used Chinese hamster Kupffer cell clones to demonstrate that two common methods of isolating transformed cell lines—either from colonies with irregular morphology or from dense foci²—can result in different degrees of karyotypic change in the isolated cell lines.

The Kupffer cell clones used in this study were characterised previously¹⁹ and after a primary cloning step remain diploid with a normal karyotype for a considerable period in culture. When these cells are infected with SV40 and transformed clones are isolated by selection of colonies with irregular morphology (colony-selected)², a high proportion of clones remains diploid for many population doublings. Although diploid, these clones display properties associated with transformation^{2,3}. In contrast, when transformed clones are isolated from foci which develop in a confluent monolayer of infected cells (focus-selected)², the clones are all heteroploid with chromosome numbers often in the hypo-tetraploid range. Differences in selective forces may explain the karyotypic variation between clones isolated by the two methods and it is probable that with the material and conditions involved, polyploidisation is not necessary for transformation of clones selected on the basis of colony morphology.

The procedure outlined in Fig. 1 was repeated for 10 untransformed Kupffer cell clones originally isolated¹⁹ from one female Chinese hamster ($2n = 22$). A previous experiment¹⁹ had demonstrated that after SV40 infection of Kupffer cells, 90% of clones isolated on the basis of irregular colony

morphology expressed SV40-tumour antigen for many population doublings. Table 1 shows pooled karyotypic data for untransformed clones, and for transformed clones after either colony-selection or focus-selection. Untransformed clones survived only up to 120 population doublings after isolation¹⁹. The two classes of transformed clone did not decline in proliferative capacity throughout the study. In all three classes of clone there was a decrease in the proportion of diploid cells as the number of population doublings increased. The major change in karyotype of untransformed clones was an increase in the proportion of hypo-diploid cells. At least 75% of cells in a cell line must possess a diploid number of chromosomes before it can be regarded as being diploid²⁰. In the untransformed clones not considered to be diploid the modal chromosome number was still 22. Isolation by colony selection yielded diploid transformed clones. As culturing proceeded there was a gradual change of karyotype in many colony-selected clones, chiefly an increase in the proportion of hyper-diploid cells. Diploid focus-selected transformed clones were not isolated and the modal chromosome number of clones isolated in this way was always greater than 22 and often approached the tetraploid range. As culturing continued the focus-selected transformed clones became more heteroploid. Chromosome abnormalities were more frequent in focus-selected transformed clones. Although infection with SV40 can stimulate successive rounds of DNA synthesis^{5,8} no endoreduplication was observed. Banding patterns in diploid colony-selected transformed clones and in diploid untransformed clones identified all chromosomes from the normal complement of both and provided no evidence of abnormalities.

Table 1 shows that the two methods of isolating SV40-transformed cells give rise to clones possessing different karyo-

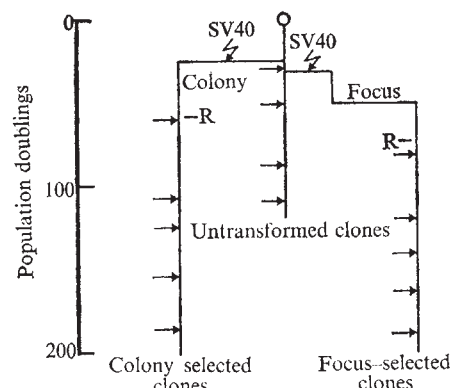


Fig. 1 Experimental outline for studying the effects of two different methods of isolating transformed cell lines on karyotype. The ordinate gives a scale of cumulative population doublings in culture since isolation of the clonal progenitor from Chinese hamster liver. The numbers of population doublings are estimates based on knowledge of plating efficiencies and the number of cells plated and recovered at each subculture. The approximate number of population doublings to form a focus at the time cells were cloned from the foci was estimated by comparing the numbers of cells in confluent dishes, with and without a known number of foci. The average estimate for doublings to form a focus from the confluent monolayer after 10 d was 13. Cells from an untransformed Chinese hamster Kupffer cell clone were infected in suspension¹⁹ with 500 plaque forming units (PFU) per cell SV40, plated at 200 cells per 9-cm dish and a colony with irregular morphology selected for continued culture. Another aliquot of the untransformed cells was infected in suspension¹⁹ with 500 PFU per cell SV40 and plated at 2×10^6 cells per 9-cm dish. Foci were allowed to form and after 10 d a single cell was cloned from one focus. The virus was a small plaque variant obtained from the Institute of Virology, University of Glasgow. The culture medium was Glasgow modified Eagle's medium supplemented with nucleosides, nonessential amino acids, pyruvate and 17% (v/v) foetal calf serum¹⁹. All clones were cultured in identical conditions kept as constant as possible and, except for the focus selection stage were subcultured when at 50% confluence. The arrows indicate stages of karyotype analysis. R, reconstruction experiments, see Table 2.

Table 1 Chromosome numbers of untransformed and SV40-transformed clones isolated by either a colony-selection or focus-selection method

	PD* since isolation of progenitor	PD since infection with SV40	Chromosome no.: proportion of cells (%)				No. diploid† clones	Metaphases‡ with abnormal chromosomes (%)	No. of clones analysed
			15-21	22	23-30	31-46			
Untransformed clones	30	—	6.4	86.4	3.6	3.6	9	1.6	10
	50	—	9.6	82.8	2.8	4.8	8	3.6	10
	90	—	21.2	69.2	4.0	5.6	4	2.8	10
SV40-transformed clones	Colony-selection								
	56	30	7.6	80.6	5.6	6.0	7	2.8	10
	106	80	17.6	67.6	7.6	7.2	5	4.4	10
	120	94	20.4	62.4	8.4	8.8	2	5.2	10
	150	124	17.2	56.4	16.4	10.0	2	5.6	10
	179	153	18.4	52.8	20.0	8.8	1	7.2	5
	Focus-selection								
	81	48	18.0	10.4	30.4	41.2	0	8.4	10
	118	85	16.8	9.2	35.6	38.4	0	12.0	10
	139	106	20.8	13.2	37.2	28.8	0	11.2	10
	165	132	24.8	8.8	33.6	32.8	0	14.4	5

For each clone 25 metaphases were examined after trypsin-Giemsa banding²¹.

* PD, population doublings.

† Clones with $\geq 75\%$ diploid cells.

‡ Includes dicentrics, minutes, acrocentric fragments and translocations.

types. Except for karyotype the two classes of transformed clone were indistinguishable. Both possessed SV40-tumour antigen as determined by immunofluorescence²¹, grew in an irregular manner to high saturation densities and could proliferate in a low serum concentration (5%). These properties² provide strong evidence that both the colony-selected and focus-selected clones were transformed by the SV40. Clones not infected with SV40 had none of these properties.

Reconstruction experiments confirmed that the method of isolating transformed clones selects for different karyotypes (Table 2). Focus-selected subclones from both a diploid and a

hypo-tetraploid SV40-transformed clone were all heteroploid, often in the tetraploid range. In contrast there was a greater proportion of diploid cells in colony-selected subclones. Thus, near-tetraploid numbers of chromosomes are presumably selected in SV40-transformed cells growing as foci. By the criteria of high saturation density, irregular culture morphology and the presence of SV40-tumour antigen, all clones isolated from the reconstruction experiments were still transformed.

These results show that the method of isolating SV40-transformed cells in culture may influence the karyotype of resulting clones and interpretation of events leading to transformation must take into account selection of only a fraction of those cells potentially available. The existence of heteroploid or hypo-tetraploid SV40-transformed clones is probably the result of selection for only a few of the karyotypes which emerge after SV40 infection. Formation of foci seems to require changes in karyotype to tetraploidy or hypo-tetraploidy and such changes may confer metabolic or proliferative advantages to cells growing in dense monolayers. In contrast, isolation of SV40-transformed cells on the basis of irregular colony morphology may select for a cell which can proliferate at very low densities. Once this capacity is realised, a clone develops with no opportunity for competition with cells of other chromosomal constitutions. While a range of different karyotypes may be compatible with such selection it is clear that diploid SV40-transformed clones can be isolated. The frequency with which diploid cells appear after selection based on colony morphology suggests that polyploidisation is not necessarily the step leading to transformation. Rather than explain the transformed nature of these diploid cells on the basis of multipolar mitoses after polyploidisation⁹ it is more attractive to suggest that polyploidisation is secondary to SV40 transformation.

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Table 2 Demonstration by reconstruction of the effect of two isolation procedures on karyotypes of SV40-transformed clones

		Chromosome no: proportion of cells (%)					
		15-21	22	23-30	21-46		
a:	Colony-selected SV40-transformed clone		10	80	4	6	
	Reconstruction:						
	Focus-selected subclones	(1)	4	16	60	20	
		(2)	4	4	48	44	
		(3)	0	16	28	56	
		(4)	12	4	52	28	
	Colony-selected subclones	(1)	20	76	0	4	
		(2)	16	72	8	4	
		(3)	40	48	17	0	
		(4)	8	80	8	4	
	b:	Focus-selected SV40-transformed clone		4	14	36	44
		Reconstruction:					
Focus-selected		(1)	8	4	24	64	
		(2)	12	12	40	36	
		(3)	4	12	32	52	
		(4)	4	20	28	44	
Colony-selected		(1)	20	12	52	16	
		(2)	20	68	12	0	
		(3)	40	52	4	4	
		(4)	12	72	8	4	

In the reconstruction experiments, one colony-selected SV40-transformed clone and one focus-selected SV40-transformed clone each provided material for the isolation of eight subclones. To obtain focus-selected subclones 200 cells from an SV40-transformed clone were mixed with 10^6 cells from an untransformed clone, plated into a 9-cm Petri dish and single cells clone from the resultant foci. To obtain colony-selected subclones 100 cells from an SV40-transformed clone were mixed with 100 cells from an untransformed clone, plated into a Petri dish containing glass fragments and those fragments carrying one colony with irregular morphology retained for further culture. Karyotype analysis was performed approximately 30 population doublings after isolation of both classes of subclone. The number of metaphases scored was 50 each for the two clones and 25 for each subclone.

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Idiotypic sharing by murine strains differing in immunoglobulin allotype

THE immunoglobulin (Ig) system is a model for studying the expression and organisation of information in a complex gene family. The expression of serologically detected determinants on the variable (V) regions of antibody molecules has been used to 'map' putative V genes. Breeding studies have suggested that V genes segregate in a Mendelian pattern and are linked to structural genes coding for constant regions of the heavy chain (C_H) of the molecule¹. Linkage analyses, however, have been based on the appearance or lack of appearance of V-region determinants in serum antibodies. The work reported here demonstrates the presence of lymphocytes with the potential to produce antibody with a V-region determinant in strains of mice reported to lack this marker in their serum immunoglobulin. The findings emphasise the necessity for examining cells outside of their natural *in vivo* environment in order to assess V-gene presence using product expression as a diagnostic marker.

A basic tool for V-gene mapping is the use of anti-idiotypic antisera to identify V-region determinants or idiotypes of the gene product. Some anti-idiotypic sera detect a single species of immunoglobulin, whereas others define closely related sets. Anti-idiotypes have been raised against populations of murine antibodies restricted in heterogeneity and homogenous myeloma proteins²⁻⁷. These antisera have been used as reagents to reveal the presence of V genes encoding the corresponding immunoglobulins. A linkage between genes coding for the V regions of heavy chains (V_H) and genes coding for C_H regions has been proposed because different V_H gene products, or idiotypes, and different C_H gene products, or allotypes, can be detected by antisera. Following appropriate genetic matings, analyses of antibodies in progeny by anti-idiotypic and anti-allotypic sera suggest that V_H genes are closely linked to their corresponding C_H genes²⁻⁸.

One commonly studied idiotypic system is the antibodies that are produced in response to phosphorylcholine (PC). The BALB/c strain of mice produces a relatively homogeneous population of antibody molecules which has the same V-region idio-type as that of the PC-binding myeloma protein, T15 (refs 5, 9-11). Although the T15 idio-type is found in BALB/c sera, it is not detected in sera of mice belonging to different allotype linkage groups^{5,12}. Observations indicate that the expression of the T15 idio-type marker is linked to the immunoglobulin C_H (Ig C_H) locus of the BALB/c strain, but it does not seem to be linked to Ig C_H loci of other strains tested. An alternative approach to using serum antibody to detect expression of idiotypes is to examine the idiotypes on antibodies produced by the clonal progeny of isolated precursors of antibody-forming cells (B cells). We have previously shown that 30% of PC-specific B cells from the AKR strain of mice produce antibody which is idiotypically indistinguishable from that produced by BALB/c mice¹³. We now show that B-cell precursors with the T15 idio-type are found in inbred strains of mice previously reported to be negative for this marker, and presumed to lack the V_H gene for the T15 idio-type.

Two anti-idiotypic sera with different specificities for T15

were prepared as reported previously. Murine anti-T15 was raised in A/He mice following the protocol of Lieberman and Humphrey¹⁴, and rabbit anti-T15 was prepared by eluting antibody from a T15-Sepharose column with PC (ref. 13). The latter antibody was inhibited by more than 80% from binding ¹²⁵I-labelled T15 in the presence of 1×10^{-3} M PC, whereas murine anti-T15 was not inhibited from binding to T15 by the presence of hapten. Thus, rabbit anti-T15 is primarily specific for the hapten-binding site of T15, and murine anti-T15 is probably specific for determinants outside this site. Both antisera were specific for the T15 idio-type in that they did not react with M167 or M603, two other PC-binding myelomas. Monoclonal antibodies were obtained from culture fluids of spleen fragments¹⁵. Spleen cells (10×10^6 - 20×10^6) from non-immune donors were injected intravenously into lethally irradiated syngeneic or histocompatible recipients that had been primed with 0.1 mg of haemocyanin 2 months earlier. The following day, spleen fragment cultures were prepared from the recipients and stimulated *in vitro* with 1×10^{-6} M 3-(azophenylphosphorylcholine)-N-acetyl-tyrosylglycylglycine-haemocyanin¹⁶. Culture fluids were collected over a period of 2 weeks and assayed for anti-PC antibodies, using anti-Fab and anti-idio-type by radioimmunoassay procedures described previously^{11,13}.

The sharing of T15 idiotypic determinants by monoclonal antibodies from mice expressing Ig C_H alleles other than those of the BALB/c strain is shown in Table 1. On a weight basis, all of the antibody that was detected with anti-Fab reacted as well as the myeloma protein T15 in inhibition assays using both hapten-inhibitable and non-inhibitable anti-idiotypes. The data indicate that some anti-PC antibodies from mice belonging to the Ig^b and Ig^d C_H allotype linkage groups are indistinguishable serologically from BALB/c antibody with respect to the T15 idio-type. As shown in Table 2, 25-70% of PC-specific B cells from non-immune mice of different strains produce antibody with the T15 idio-type. Using a radioimmunoassay to detect the Ig-2^a allotype, we confirmed the lack of association between the T15 idio-type and BALB/c allotype. All of the BALB/c clones that produced IgA had the Ig-2^a allotype, whereas none of the T15-positive IgA antibody from C57BL/10, CB-20, B10.D2 and CAL-20 had this allotype.

Previous linkage studies of serologically-identified V_H and C_H regions have been based on the appearance of an idio-type in serum antibody from mice immunised with a variety of antigens²⁻⁸. A tentative genetic map has been constructed, placing certain V_H genes relative to one another and the C_H loci¹⁸. Interpretation of idiotypic data from heterogeneous serum immunoglobulins, however, must be made with caution: detection of low levels of an idio-type may indicate either weak cross reactions among a heterogeneous population of antibodies, none of which is identical to the idio-type, or the presence of the relevant idio-type as a minor population. Also, serum antibody

Table 1 T15 idio-type on some monoclonal antibodies selected from several murine strains

Strain	Ig C_H allotype linkage group	Antibody in culture fluids detected by		
		Anti-Fab (ng)	Murine anti-T15 (ng)	Rabbit anti-T15 (ng)
BALB/c	a	8.6	9.0	10.0
C57BL/10	b	22.0	22.0	21.0
B10.D2	b	2.5	2.8	2.0
CB-20	b	13.0	13.0	10.8
CAL-20	d	13.2	16.5	11.6

Antibody was quantified using T15 protein as the standard. Murine anti-T15 is a hapten non-inhibitable anti-idio-type, and rabbit anti-T15 is a hapten-inhibitable anti-idio-type. Strain B10.D2 is congenic with C57BL/10 but has the histocompatibility locus of BALB/c; CB-20 is congenic with BALB/c but has the Ig^b C_H allotype; and CAL-20 is congenic with BALB/c but has the Ig^d allotype.

Table 2 Frequency of T15 precursors in allotypically different strains

Strain	Total PC-specific precursors per 10 ⁶ B cells*	Precursors with T15 idiotype per 10 ⁶ B cells	% PC-specific precursors with T15 idiotype	% clones making IgA with Ig-2 ^a allotype†
BALB/c	23.2	16.7	72	100
CB-20	19.2	11.4	59	0
CAL-20	29.4	19.8	67	0
B10.D2	6.5	2.2	34	0
C57BL/10	15.8	4.0	25	0

*160 × 10⁶ donor spleen cells from each strain were analysed in the splenic focus assay. The frequency of precursors was calculated using donor cell homing and stimulation efficiencies previously reported¹⁷.

†Clones making IgA were identified using ¹²⁵I-labelled rabbit anti-IgA to detect antibody bound to PC-immunoadsorbent. Anti-allotype sera specific for Ig-2^a determinants on the α C_H region were raised in A/He mice using S117 IgA protein. Antibody with this allotype was detected by adding a 33% (NH₄)₂SO₄ fraction of anti-allotype sera to antibody bound to PC-immunoadsorbent and then adding ¹²⁵I-labelled rabbit anti-IgG2. Anti-allotype sera did not react with BALB/c IgG1 or IgM antibody, but identified BALB/c IgA antibody with or without the T15 idiotype equally well.

represents the expression of an antigen-selected population of B cells, so absence or low levels of an idiotype may not be related to the actual repertoire of B-cell V genes.

The data presented here indicate that a substantial number of precursors for T15 antibody-forming cells are present in non-immune spleens of mice that do not seem to express this idiotype in their circulating antibody. The isolation and stimulation of B cells in the splenic focus assay of Klinman¹⁵ has proved to be an effective technique for: identifying homogeneous antibody that is produced by single clones of cells; determining the frequency of precursors of antibody-forming cells; and detecting minor populations of B cells which may not be stimulated by antigen *in vivo*. In this regard, the analysis of individual non-immune B cells is one of the most effective methods for determining whether a particular V gene is present, based on expression.

The detection of T15-positive clonal precursors in serum-negative strains has two important implications. First, the repertoire of V genes expressed by B cells may be larger than the sum of specificities that are readily detected in selected populations of serum antibody. The differential expression of myeloma proteins in two inbred strains of mice also suggests that only a fraction of the antibody repertoire may be expressed, because of regulatory phenomena rather than strain-specific differences in V structural genes¹⁹. Thus, there may be greater V-gene diversity than would be predicted from serum immunoglobulin estimates. Second, if idiotypes accurately reflect structural genes, previously constructed genetic maps of V_H loci may not be valid. An alternative explanation for the interesting correlation of idiotype to C_H allotype in serum immunoglobulin is that the control of phenotypic expression of an idiotype *in vivo* may be linked to the C_H loci²⁰. This 'control' may, of course, in part be exercised by other V_H genes themselves.

The interpretation of any serological assay, however, is limited compared with the use of protein amino acid sequences to prove identity of gene products. Such a structural analysis may be possible using new techniques of cell fusion of normal antibody-producing cells and myeloma cells to generate large amounts of homogeneous protein for chemical studies²¹. Alternatively, the presence of a particular V gene could be assessed in the bulk DNA in T15-negative strains by hybridisation techniques using a cDNA probe made from mRNA of the T15 myeloma.

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Antigen-antibody complexes elicit anti-idiotypic antibodies to self-idiotopes

THE variable (V) regions of each immunoglobulin molecule carry unique antigenic determinants (idiotopes, Id)^{1,2}. Anti-Id antibodies recognise not only the antibody, but also the V regions of antigen receptors on both T and B lymphocytes^{3,4}, and it has been postulated that auto-anti-Id antibody^{5–10} (anti-antibody) may be an important homeostatic mechanism in immunoregulation^{11–13}. An essential prerequisite for such a mechanism is that idiotype determinants should be highly autoimmunogenic. In fact, although it has proved relatively easy to induce anti-Id antibodies by immunising allogeneic or xenogeneic individuals with immunoglobulins, induction of auto- or iso-anti-Id antibodies usually requires prolonged hyperimmunisation using potent adjuvants^{7,8,10}. Recently, I showed that antibody, given as an antigen-antibody complex, acts as a very effective adjuvant for inducing immunological memory for antibody production^{14,15}, and show here that antigen-antibody complexes also enhance the production of anti-antibodies.

The antibody used in these experiments was M315, which is an IgA (α, λ) myeloma protein of BALB/c mice with anti-dinitrophenyl (DNP) antibody activity¹⁶. BALB/c mice were given a single injection of preformed immune precipitates of dinitrophenylated haemocyanin (DNP-KLH) and M315 intravenously. Complexes were made at varying (w/w) ratios of antibody to antigen, previously determined by radioimmunoassay (using ¹²⁵I-labelled DNP-KLH), and spectrophotometric analysis of precipitates. After three weeks the mice received a booster dose of 100 μg purified¹⁷ M315 intraperitoneally, and were bled 10 d later.

Individual sera were tested by a double-antibody radioimmunoassay¹⁸, using ¹²⁵I-labelled M315 as antigen. The results (Fig. 1) show that all mice given DNP-KLH-M315 complexes developed substantial titres of anti-M315 antibodies. In those given 10 μg DNP-KLH plus M315, complexes made with the highest ratio of antibody to antigen were the most immunogenic. DNP-KLH alone, or M315 alone elicited barely significant antibody titres.

Figure 2 compares the specificity of the antibodies from a representative group (group 3, Fig. 1) given antigen-M315

complexes, with that of anti-Id antibodies raised by hyperimmunisation with purified M315 in adjuvant¹⁰. Binding of both lots of antibody was totally inhibited by ~10-fold excess of M315, but not significantly inhibited by ~100-fold excess of four other BALB/c IgA myelomas, nor by M104E (μ , λ), normal mouse IgG or normal BALB/c serum. Antibodies raised by both procedures are therefore anti-idiotypic. However, while hyperimmunisation with M315 induces antibodies which are totally inhibited by free hapten (DNP-lysine), those made in response to antigen-M315 complexes were only partially inhibited by hapten. It is well established that the hapten-binding site is the major idiotype determinant on M315 recognised by BALB/c mice¹⁹. However, mice given M315 (in adjuvant) with blocked combining sites respond to other idiotype determinants on the protein¹⁸. Clearly then, mice given the antibody in complex with antigen make both 'site-directed' and 'non-site-directed' anti-idiotypic antibodies, as might be predicted.

These results thus show that microgram quantities of antibody complexed with antigen induce anti-Id antibody to self-idiotype in the absence of extrinsic adjuvant. Indeed, the antibody titres elicited by this simple manoeuvre are comparable with those obtained by hyperimmunising mice with purified M315 in Freund's adjuvant, following the procedure of Sakato and Eisen¹⁰ (data not shown). Kelus and Gell²⁰ first used antigen-antibody complexes to induce anti-Id antibodies in allogeneic rabbits, although they failed to induce autoantibodies. I have argued elsewhere¹⁴ that the immunogenicity of antigen-antibody complexes is due to their rapid localisation in lymphoid follicles²¹. This would explain why complexes of myeloma proteins with xenogeneic or allogeneic IgG⁸, or of DNP-coupled myeloma protein and anti-DNP antibody⁹, readily induce iso-anti-Id antibody formation; all these procedures would favour follicular localisation of the antibody.

Fig. 1 Induction of anti-idiotypic antibody production with antigen-antibody complexes. Dinitrophenylated haemocyanin (DNP-KLH, containing 200 DNP groups per 10⁶ molecular weight protein) and serum from mice carrying the MOPC315 tumour were mixed and incubated at 37 °C (30 min) and then at 4 °C (overnight). Immune precipitates were washed, resuspended and injected intravenously into groups of 3-month-old BALB/c mice. Thus, mice received either 10 or 100 μ g DNP-KLH; Ab:Ag ratios refer to w/w ratios of antibody:antigen in complexes, determined previously. Group 9 received an amount of M315 (alone) equivalent to that given to group 8. Mice in group 10 were left untreated. All groups received booster doses of 100 μ g purified M315 intraperitoneally on day 21 and were bled on day 31. Antibody titres were determined by radioimmunoassay using 2 μ l antiserum, 30 ng ¹²⁵I-labelled M315 and 100 μ l goat-anti-mouse IgG antiserum. Bars represent means $\pm$ s.e.m. ($n = 5$).

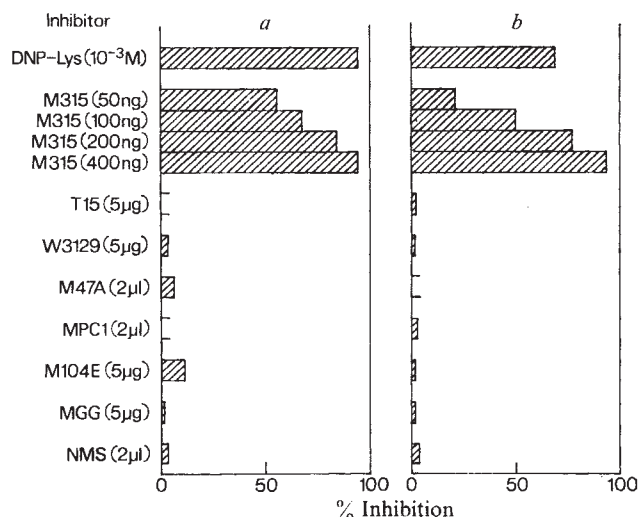
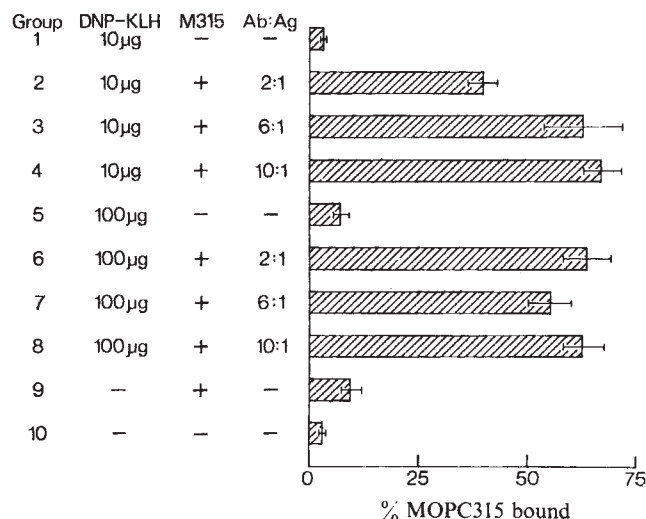


Fig. 2 Idiotype specificity of anti-M315 antibodies elicited *a*, by hyperimmunisation with M315, or *b*, by giving DNP-KLH-M315 complexes. In *a*, the serum was from 10 mice given purified M315 first in complete Freund's adjuvant, then in incomplete adjuvant, and subsequently in saline, according to Sakato and Eisen¹⁰. In *b*, sera from mice in group 3 (Fig. 1) were pooled (other groups gave comparable results). In each case 2 μ l of the antiserum was incubated with 10⁻³M DNP-lysine, or various myeloma proteins, as indicated, for 30 min at 37 °C before addition of 30 ng ¹²⁵I-labelled M315 as in Fig. 1. The inhibitors were M315 (α , λ), T15 (α , κ), W3129 (α , κ), MPC1 (α , κ), M47A (α , κ), M104E (μ , λ), MGG (normal mouse IgG) and NMS (normal BALB/c serum). MPC1 and M47A were added in the form of serum from tumour-bearing mice, while the other proteins were immunochemically pure.

The present results are compatible with network theories of immunoregulation¹¹⁻¹³, as they suggest that antibodies only acquire marked autoimmunogenicity when complexed with the eliciting antigen. It remains to be determined if this mechanism also applies to major idiotypes; for example, the T15 idiotype¹³, which dominates the response of BALB/c mice to phosphorylcholine and which might be less autoimmunogenic than a minor idiotype such as M315. It could be argued that the doses of antigen-idiotype complexes used in the present study are higher than those available during a normal immune response. However, we do not know what fraction of the injected immune complex actually reaches immunocompetent cells (as much of it is undoubtedly degraded by macrophages), nor what levels of anti-Id antibodies are required to exert an immunoregulatory role *in vivo*.

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Molecular variants of human Ia-like antigens

THE Ia antigens, a group of plasma membrane proteins predominately expressed on B lymphocytes and thought to be products of the 'immune response (*Ir*) region' of the major histocompatibility complex¹⁻⁴, are believed to play a part in the mechanism of T-B cell cooperation¹⁻⁵, stimulation in mixed lymphocyte culture¹, graft-versus-host reactions¹ and T-cell suppression⁶. They have been demonstrated on the surface of a variety of cells which take part in intercellular interactions, such as B lymphocytes, macrophages, suppressor T cells, sperm⁷ and Langerhans cells of the skin⁸. In several species these glycoproteins exist in a complex containing 33,000 and 27,000 molecular weight (MW) moieties^{9,10}. Although Snary *et al.*¹¹ have shown that the human alloantigens may reside predominantly on the 33,000 MW chain, alloantisera are able to precipitate both components. Attempts to show molecular variants within either chain by immune precipitation with alloantisera have given inconsistent and varying proportions of the light and heavy chains¹². Springer *et al.*¹³ used two-dimensional gel electrophoresis of human B-cell alloantigens to show the association of the two chains, but observed smearing in the isoelectric focusing dimension which they attributed in part to aggregation. We describe here the preliminary successful subfractionation of the 33,000 MW chain of human Ia-like antigens by a modification of the two-dimensional electrophoresis system which combines isoelectric focusing and discontinuous sodium dodecyl sulphate (SDS) electrophoresis.

The *Ir* region of the mouse has now been divided serologically and genetically into at least five *Ir* subregions¹⁴. At least two distinct genetic loci for the human B cell alloantigens have been described using family studies in which one member is an HLA recombinant^{15,16}. Detection of other loci are restricted in man by the rarity of crossover events between these closely linked genes. Our technique for separating these antigens could facilitate the designation of specific B-cell alloantigens to distinct genetic regions.

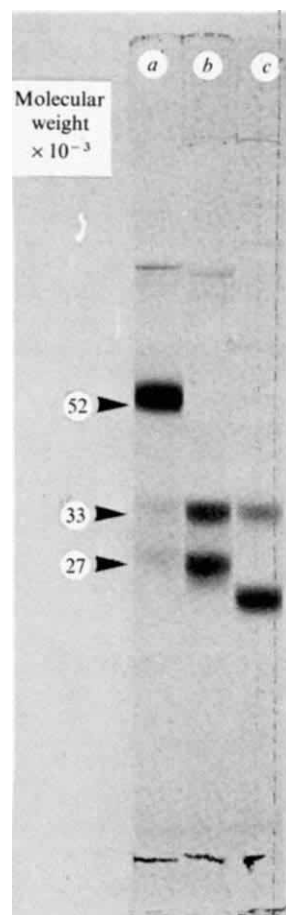
The isolation and characterisation of the B-cell antigens and the antisera raised against them are detailed elsewhere^{8,17}. Briefly, live cells from cultured human B lymphoblastoid long-term cell lines or isolated from patients with chronic lymphocytic leukaemia (CLL) were surface radioiodinated by the lactoperoxidase method and then extracted into 1% or 0.5% Triton X-100 respectively. This extract was filtered through a Biogel A1.5 column, and the peak showing the greatest enrichment of isotope activity was passed down a Sepharose-concanavalin A affinity column. The fraction eluted with α -methyl mannoside contained the B-cell antigens as the major bands (33,000 and 27,000 MW) with 3-6 minor protein bands on discontinuous SDS gels (Fig. 1). No β_2 microglobulin was precipitable, nor were any bands corresponding to HLA antigens or immunoglobulins present in these preparations. In human B-cell alloantigens¹³, the two chains are non-covalently bound and are dissociated in SDS only after boiling. Incubation in SDS at 37 °C and subsequent reduction results in a predominantly undissociated complex at 52,000 MW (Fig. 1a). After boiling in SDS for 2 min before reduction, the molecule dissociates completely into subunits of MWs of 33,000 and 27,000 (Fig. 1b). In non-reducing conditions the lighter of the two chains has an apparent MW of 22,000 (Fig. 1c), suggesting the presence of an intrachain disulphide bridge which is broken by reduction with consequent slowing of migration due to some unfolding of the subunit. These gels show that the two major chains subsequently examined in the two-dimensional system do arise from the major 52,000 MW complex and not from other minor impurities.

The isolated B cell antigens were examined by the two-dimensional gel system of O'Farrell¹⁸ with modifications in the ampholyte system (outlined in the legend to Fig. 2). This

technique uses isoelectric focusing in urea in the first dimension, and discontinuous SDS gel electrophoresis in the second. Several distinct spots at a molecular weight corresponding to the 33,000 MW chain of the B-cell antigens can be seen (Fig. 2). They are clustered within a narrow pH range (pH 5.3 to 5.4) on each of the gels shown. The B lymphoblastoid cell line NC-37 yields a consistent cluster of 4 spots at 33,000 MW of comparable staining intensity with Coomassie blue (Fig. 2a). A fifth and possibly a sixth spot of variable staining intensity, on either side of the cluster has been observed occasionally on other gels of this cell line. Some spots within the NC-37 cluster show slight yet reproducible differences in mobility in the SDS dimension. The relative quantity of each spot varies with different cell lines, but for each cell line the patterns and intensities are reproducible.

We have also analysed antigens isolated from CLL cells, on which the expression of B-cell antigens has been reported^{17,19,20}. The intact cells were first radioiodinated by the lactoperoxidase technique to label surface glycoproteins. Double immunoprecipitates using rabbit antisera raised to the isolated human B-cell antigens¹⁷ were obtained from the isolated CLL antigens, solubilised and electrophoresed in the two-dimensional system already described. The autoradiograph revealed one major and three minor radioactive spots clustered at MW 33,000 (Fig. 2b). No differences in mobility between the spots in the SDS dimension were seen. This pattern differs from that seen in the cell line NC-37, but is very similar to that seen in autoradiographs of radiolabelled antigens isolated from another B cell line, Daudi (not shown). The major spots of both the CLL and

Fig. 1 Discontinuous SDS 10% acrylamide gels of Ia-like antigens isolated from the human B lymphoblastoid line NC-37. Samples were prepared in 2% SDS, 0.0625 M Tris pH 6.8, and then (a) reduced in dithiothreitol (DTT) for 30 min at 37 °C, (b) boiled for 2 min before reduction or (c) boiled for 2 min and not reduced.



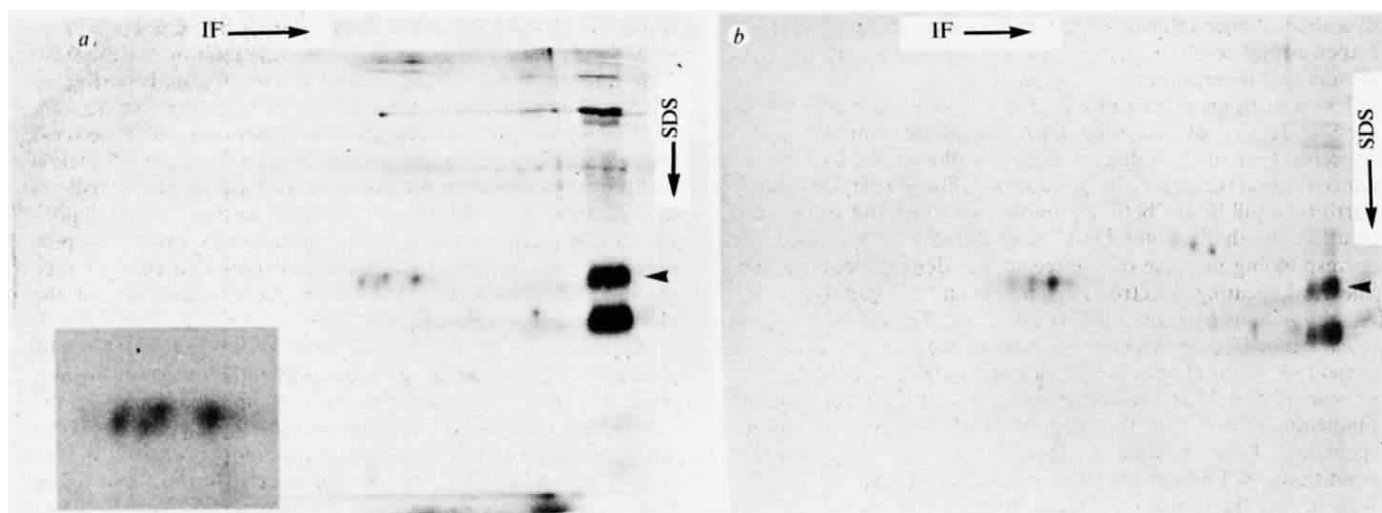


Fig. 2 *a*, Isolated B cell antigens from the human lymphoblastoid cell line NC-37 showing a cluster of spots at 33,000 MW. Insert shows an enlargement of the cluster. Gel was stained with Coomassie blue. *b*, Autoradiograph of B cell antigens precipitated from an extract of chronic lymphocytic leukaemia cells by a rabbit antiserum to antigens purified from a B lymphoblastoid line. A cluster of 7 surface-labelled glycoproteins is seen at 33,000 MW. Two-dimensional gels according to O'Farrell with the following modifications: Triton X-100 used instead of NP-40, and 2% ampholytes, pH range 4-6, 5-7, 2-11 in a 2:2:1 ratio. The B cell antigens were separated by isoelectric focusing in the presence of urea in the first dimension; gels were equilibrated with SDS sample buffer for 30 min, then laid on top of a 10% polyacrylamide discontinuous SDS slab gel in the second dimension. In the second dimension (SDS), samples of the antigens were run in parallel on the right side of the gel for direct MW comparisons. The arrow indicates the 33,000 MW chain in the second dimension. The alkaline end of the isoelectric focusing gel is to the right.

Daudi antigens are in the same position within the cluster. The differential staining observed in the NC-37 antigens, which is consistent on autoradiographs, and the differential labelling seen in the CLL antigens may reflect relative concentration differences among the species represented by the spots. However, the possibility of relative differences in the accessibility to enzymatic radioiodination and in the reactivity with the heterologous antisera must be borne in mind. The triplet cluster of 3 additional spots of slightly higher MW and more alkaline *pI* is reproducible. Its potential significance as a marker for the leukaemic state will warrant further study.

The modifications of the two-dimensional gels outlined in this paper give a clear resolution of the 33,000 MW chain. The alkaline end of the isoelectric focusing gel reaches slightly above pH 6.1. For this reason, the 27,000 MW chain, which has a *pI* slightly above this range, is not well represented in the gels shown. However, gels with a slightly higher pH range are capable of reproducibly resolving the 27,000 MW chain into at least two components.

The B-cell allospecificities have been tentatively assigned to the 33,000 MW chain¹¹. We have observed a reproducible separation by charge of this chain by two-dimensional electrophoresis in several B-cell lines and in CLL. Whether this charge separation results from differences in carbohydrate composition, amino acid sequence or the improved method of antigen preparation is currently under investigation. This technique of separation, in combination with antigen preparations from selected B-cell lines and the corresponding alloantisera, makes it possible rapidly to analyse and map molecular variations within this complex. The comparison of different B-cell lines, leukaemic cells and other isolated subpopulations of cells which bear these antigens, such as suppressor T cells, macrophages, and Langerhans cells, could result in further functional and chemical definition of the Ia alloantigen system.

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Lipid-dependent and phloretin-induced modifications of dipicrylamine adsorption by bilayer membranes

THE hydrophobic anions of dipicrylamine (DpA⁻) and of sodium tetraphenylborate (TphB⁻) have been shown to adsorb at deep potential minima near the surfaces of lipid bilayer membranes¹. Use of a high field voltage-jump technique has led to direct measurement of the membrane surface charge density attributable to adsorption of these anions^{2,3}, and analyses of such measurements have shown that the magnitude of the membrane surface potential resulting from adsorption of both DpA⁻ and TphB⁻ is too large to be accounted for by conventional Gouy-Chapman theory^{4,5}. Crucial to an understanding of this discrepancy is the question of the location of the plane of

adsorbed charge relative to the membrane-solution interface. Experimental results reported here are consistent with the conclusion that these planes are coincident.

Using methods described elsewhere^{2,6}, we have measured the surface density of adsorbed DpA^- as a function of DpA^- concentration in the adjacent aqueous phases. At low DpA^- concentration the observed relationship is linear, reflecting simple partition equilibrium between anions adsorbed and in aqueous solution. As the aqueous DpA^- concentration is increased, the corresponding increase of surface charge density becomes sub-linear, indicating electrostatic repulsion of impinging hydrophobic ions by those already adsorbed. The transition from linear to sublinear dependence should take place when the magnitude of the change in surface potential due to adsorption is of order (kT/e) . Here k is Boltzmann's constant, T is the absolute temperature, and e is the magnitude of the electron charge. Applying Gouy-Chapman theory⁷ in our experimental conditions—0.1 M uni-univalent electrolyte in water at 25 °C—we find that the transition surface charge density corresponding to this potential is $1.93 \mu\text{C cm}^{-2}$. Transition surface charge densities measured in these conditions are lower by a factor of more than two, reflecting the discrepancy noted above.

In Fig. 1 surface density of adsorbed charge is plotted against aqueous phase concentration of DpA^- . Data are presented for bilayer membranes formed from dioleoyl phosphatidylcholine (PC), bacterial phosphatidylethanolamine (PE), glycerol monooleate (GMO), and two GMO-cholesterol mixtures. The curves can be brought into coincidence by horizontal shifts, so it may be inferred that the principal difference among the lipids studied is in the coefficient β characterising partition equilibrium¹ between DpA^- ions adsorbed and in solution. Principal features of these data are: (1) transition surface charge densities, as determined by intersecting dashed lines extrapolated from the curves, are the same for all lipids and lipid mixtures used; and (2) the data for GMO and its mixtures with cholesterol are well fit by a single curve, indicating minimal variation of partition equilibrium with addition of cholesterol.

Data for PC membranes illustrate the effect of phloretin addition to the aqueous phases (Fig. 2). The primary effect is the reduction of the partition coefficient; observed transition surface charge densities are unmodified by addition of phloretin.

As current reviews^{8,9} make clear, boundary potentials in membranes involve both diffuse double layer potentials, gen-

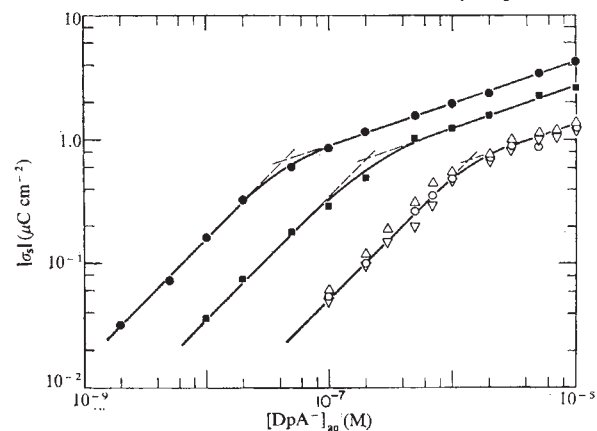


Fig. 1 The magnitude of σ_s , the surface density of adsorbed charge, plotted against the aqueous phase concentration of DpA^- . Curves are shown for different lipids and lipid-cholesterol mixtures. Mole fractions of lipid and of cholesterol in the membrane-forming solutions are indicated for the mixtures. Linear ranges of the curves are indicated by lines of unit slope on these log-log plots. Sublinear ranges are fit by lines of slope 1/3; theoretical justification of this power law dependence is given elsewhere^{4,6}. The intersection of these line segments, projected on to the $|\sigma_s|$ -axis, determines the transition surface charge density for each curve. NaCl concentration, 0.1 M, temperature 25 °C. ●, Phosphatidylcholine ($\beta = 0.16 \text{ cm}$); ■, phosphatidylethanolamine ($\beta = 3.6 \times 10^{-2} \text{ cm}$); ○, glycerol monooleate (GMO); △, GMO:cholesterol 0.7:0.3; ▽, GMO:cholesterol 0.5:0.5. For GMO-containing systems, $\beta = 5.2 \times 10^{-3} \text{ cm}$.

erated by charged interfaces, and inherent dipole potentials as well. The latter act to make the membrane interior positive with respect to the surface. The interiors of both PC and PE are more positive than that of GMO, by 120 mV (ref. 10) and 130 mV (refs 11,12) respectively. Furthermore, incorporation of cholesterol into bilayers increases their positive interior potential^{13,14}, while addition of phloretin to the surrounding aqueous phases reduces this potential^{15,16}. In discussing the location of the DpA^- adsorption plane we will refer to 'interior' and 'exterior' dipole potentials, meaning that the associated potential changes take place on the membrane side or on the solution side of the adsorption plane respectively.

The variations of partition coefficient shown in Fig. 1 could be attributed either to differing chemical affinities of the adsorption planes for DpA^- or to differing exterior dipole potentials in the systems studied. If the latter were most important, then the adsorption planes of PC and PE would be more positive than that of GMO by 90 mV and 50 mV respectively. Lack of agreement with the relative potentials cited above, particularly for PE, indicates that the spatial relationship of inherent dipole layers to the adsorption plane in pure lipids cannot be inferred from these data. The change of dipole potential accompanying incorporation of cholesterol into GMO membranes, however, clearly takes place interior to the adsorption plane. This is shown by constancy of the partition coefficient. Similar results have been obtained in other studies involving addition of cholesterol^{14,17}. The interior dipole potential does indeed increase with cholesterol addition, by about 80 mV at the highest mole-fraction used here, as indicated by a 20-fold decrease of transient current relaxation time at low applied field (see also ref. 14).

Although strictly chemical effects cannot be excluded, the monotonic decrease of partition coefficient with phloretin addition (Fig. 2) suggests development of an exterior dipole layer. The implied decrease of potential at the adsorption plane would be consistent with other observations^{15,16}.

To remove the discrepancy with Gouy-Chapman theory noted at the outset, a three-capacitor model has been proposed^{5,12}. In this view the inner (geometric) capacitance of the membrane is in series with two outer capacitors representing the double layers resulting from anion adsorption near the membrane surfaces. At high ionic strength, charge separation on each outer capacitor is attributed to a charge-free layer in the lipid separating the anion adsorption plane from the diffuse counterion atmosphere in the adjacent water. This separation accounts for the increased magnitude of potential at the adsorption plane.

An alternative approach^{4,6} postulates that the water adjacent to the adsorption plane is in a state of dielectric saturation. Thus a sufficient surface charge density produces a more intense double layer electric field, hence an increased magnitude of surface potential.

For both models any variation of the partition coefficient alone would produce horizontal displacement of theoretical curves drawn to fit the data of Figs 1 and 2. Thus any exterior dipole potential operative in the systems studied here would simply modify the partition coefficient by a fixed Boltzmann factor, resulting in such a displacement. Hence only the change of potential at the adsorption plane due to anion adsorption is pertinent to the transition from linear to sublinear dependence. The three-capacitor model predicts that the transition occurs when this potential change, taking place across the charge-free layer, is of the order (kT/e) . The corresponding surface density of adsorbed charge would then depend on the effective thickness and dielectric constant of the layer, that is, on its specific capacitance. For the dielectric saturation model, on the other hand, this potential change and associated transition surface charge density are determined solely by aqueous dielectric properties and the indifferent electrolyte concentration in the water adjacent to the adsorption plane.

All of the data presented here can be fit to the three-capacitor model using the indicated partition coefficients for each curve, together with a single outer capacitance value of $44 \mu\text{F cm}^{-2}$ ($\pm 10\%$). The data also fit the dielectric saturation model using the

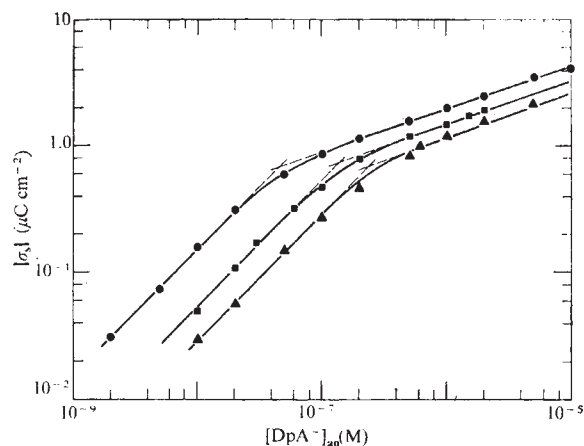


Fig. 2 The surface density of adsorbed charge plotted against aqueous phase concentration of DpA^- for PC membranes. Fixed amounts of phloretin were added to the aqueous phases as indicated. All experiments were performed in unbuffered solutions, with pH ranging between 5.6 and 5.0. In these conditions neutral phloretin is the species responsible for modification of membrane dipole potential^{1,5}. Temperature 25 °C; NaCl concentration 0.1 M. ●, No phloretin added ($\beta = 0.16$ cm). ■, Phloretin 2×10^{-6} M added ($\beta = 5.7 \times 10^{-2}$ cm). ▲, Phloretin 6×10^{-6} M added ($\beta = 3.0 \times 10^{-2}$ cm).

indicated β values together with a single transition surface charge density value of $0.68 \mu\text{C cm}^{-2}$ ($\pm 10\%$). Despite this ambiguity, two significant points favour the dielectric saturation model. First, insensitivity of the transition surface charge density to membrane lipid composition and to added phloretin is readily understood; this parameter is determined solely by properties of the adjacent aqueous phases which were not varied in these experiments. The corresponding insensitivity of the outer capacitance, as envisaged by the three-capacitor model, would have to be regarded as fortuitous since this parameter would be sensitive to the structure and dielectric properties of the various lipids and lipid mixtures, and possibly also to adsorbed neutral phloretin. The second point is the unambiguous demonstration that the dipole potential change accompanying introduction of cholesterol into GMD membrane takes place interior to the DpA^- adsorption plane. Both points are consistent with the conclusion that this plane is coincident with the membrane-solution interface.

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Pulmonary bronchiolar alkylation and necrosis by 3-methylfuran, a naturally occurring potential atmospheric contaminant

A REPORT¹ from the National Naval Research Laboratory has indicated that 3-methylfuran (3MF) was a major atmospheric contaminant during a smog alert in Washington DC in August 1973. Although the source of the 3MF was unknown, the authors suggested that it could be formed from atmospheric photooxidation and degradation of volatile hydrocarbons such as isoprene and other terpenes. These naturally occurring precursors are released in large amounts² into the atmosphere by vegetation, and may be concentrated during certain weather conditions; deciduous forests, such as those covering the Appalachian Mountains, are thought to be a major source^{2–4}. The suggestion that 3MF, which apparently is not present in automobile exhaust emissions, could be a major contributor to certain urban smogs aroused considerable controversy, for automobiles have been widely considered to be the major source of atmospheric pollutants, especially in nonindustrial areas such as Washington DC. We have found no toxicological data concerning 3MF, although many other furans are highly toxic to the liver, kidneys^{6,7} and lungs^{7–9} of experimental animals, after metabolism (catalysed by cytochrome P-450-dependent mixed-function oxidases) to highly reactive alkylating agents within the target tissues. For example, when 4-ipomeanol^{8,9} is given to experimental animals by intraperitoneal, intravenous or oral routes, it is highly toxic to pulmonary nonciliated bronchiolar cells (Clara cells)⁹, which are an important locus of cytochrome P-450-dependent mixed-function oxidase (MFO) enzymes in the lung. The nonciliated bronchiolar cells are therefore important potential target cells for environmental toxins or carcinogens which require MFO-mediated activation⁹. We report here that 3MF is metabolised *in vivo* in mice to a potent alkylating agent which produces acute pulmonary bronchiolar necrosis. These results suggest that 3MF could play a significant part in the pathogenesis of human lung disease.

Figure 1 shows that 3MF, like 4-ipomeanol^{8,9}, is converted by P-450-dependent MFO microsomal enzymes isolated from mouse lungs, to a highly reactive metabolite which alkylates the microsomal protein. Figure 2 and Table 1 show that metabolic activation of 3MF occurs similarly *in vivo*, and that the alkylating metabolite of 3MF is highly toxic to the lung

Table 1 Effect of intraperitoneal administration of piperonyl butoxide on the pulmonary bronchiolar alkylation and toxicity of 3MF given to mice by inhalation*

Group†	Covalent binding of 3MF to lung macromolecules‡ (nmol bound per mg lung protein) $\pm$ s.e.m.	Bronchiolar necrosis**
Control§	3.9 (± 0.3)	++ +
Pretreated with piperonyl butoxide	2.0 (± 0.1)¶	+

*1-h exposure to atmosphere containing 0.23 mmol 3-MF-[methyl-³H] (specific activity 12 mCi per mmol) per litre of air; all animals sacrificed 24 h after exposure.

†Each group consisted of 5 NIH-Swiss mice, each weighing 10 g.

‡Covalently bound radioactivity measured as described elsewhere⁹.

§Each control mouse received 0.1 ml of corn oil 1 h before experiment.

||Piperonyl butoxide (1,600 mg per kg) given intraperitoneally as a solution in corn oil (0.1 ml final volume per 10 g mouse), 1 h before experiment.

¶Significantly less than control value ($P < 0.01$).

**Lesions scored according to degree of involvement of larger compared to smaller airways; (++) indicates bronchiolar necrosis visible in all but the largest, cartilaginous airways; (+) indicates bronchiolar necrosis visible only in smallest, terminal bronchioles.

bronchioles. Figure 2b gives a representative example of autoradiograms of the lungs of mice given labelled 3MF either intraperitoneally or by inhalation. Both alkylation and necrosis were most pronounced in the smaller bronchioles. In many instances, terminal bronchioles were almost completely denuded of nonciliated cells, leaving behind a thin layer of abnormal looking ciliated cells. The lesions produced by 3MF and 4-ipomeanol appear to be very similar, if not identical, but further studies are underway to compare them at an ultrastructural level.

Intraperitoneal administration of piperonyl butoxide, an inhibitor of the MFO-mediated microsomal activation of 3MF (Fig. 1), prevented both the bronchiolar alkylation and the bronchiolar necrosis of 3MF given by inhalation (Table 1). These results are consistent with the view that a metabolite of 3MF is formed *in situ* that alkylates the lung bronchioles, leading to their necrosis.

If 3MF causes severe bronchiolar disease in experimental animals, it seems possible that during atmospheric conditions in which 3MF is formed and sufficiently concentrated in the atmosphere, the compound could contribute to the exacerbation of acute or chronic pulmonary bronchiolar diseases in man. Moreover, since many alkylating agents are also carcinogenic, the possibility that 3MF can induce bronchial neoplasia must also be considered. It will be important to confirm the presence of 3MF as an atmospheric contaminant and to determine if temporal and/or regional differences in its levels are sufficient to permit epidemiological evaluations of its public health significance. It seems unlikely that 3MF is an atmospheric contaminant unique to the Washington, DC area.

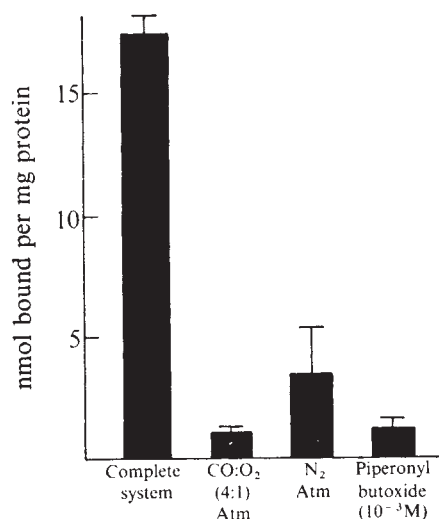


Fig. 1 NADPH-dependent covalent binding of radioactivity from 3-methylfuran-[methyl-³H] in mouse lung microsomes (synthesis of radiolabelled 3MF to be reported elsewhere). NIH-GP mice were sacrificed by cervical dislocation and lung microsomes prepared according to standard procedures⁸. Incubations were run in triplicate for 10 min at 37 °C, using a total volume per incubation of 2 ml of phosphate buffer (pH 7.4) containing 4 mg of microsomal protein, 2 μmol of ³H-MF (specific activity, 0.25 mCi mmol⁻¹), and an NADPH generating system, consisting of 5 μmol NADP, 15 mmol glucose-6-phosphate and 1 unit glucose-6-phosphate dehydrogenase. Incubations were run under an air atmosphere unless otherwise indicated. Reactions were terminated by the addition of 1 ml of 10% trichloroacetic acid, and the microsomal protein assayed for covalently bound radioactivity as described elsewhere^{8,9}. No enzyme-catalysed binding was observed in the absence of NADPH. Microsomal alkylation also required oxygen, and it was strongly inhibited by a carbon monoxide-enriched atmosphere. Addition to the incubation medium of piperonyl butoxide, a well known inhibitor of drug metabolism, caused a marked decrease in microsomal alkylation. These results are consistent with the view that mouse lung microsomes convert 3MF to an alkylating metabolite by means of cytochrome P-450-dependent oxidation.

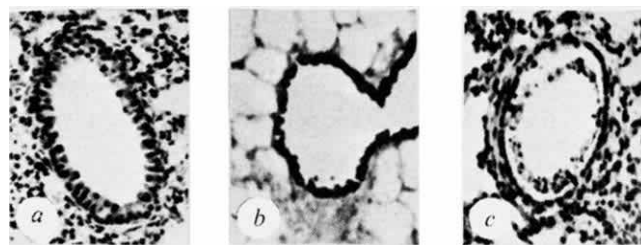


Fig. 2 Photomicrographs (magnification ×200) showing bronchiolar alkylation and necrosis by 3MF. *a*, A normal mouse bronchiole; *b*, a representative autoradiogram showing the extremely intense, and preferential accumulation of covalently bound 3MF-metabolite (accumulations of black photographic granules) in terminal pulmonary bronchioles of the mouse. Essentially identical autoradiograms were obtained from mice given [³H]-3MF (12 mCi per mmol specific activity) by intraperitoneal administration, or by inhalation. Animals were sacrificed 8 h after exposure and the lungs were subjected to a procedure described elsewhere⁹ for the autoradiographic localisation of covalently bound metabolite. *c*, An example of bronchiolar necrosis seen in lungs of mice 24 h after the administration of 3MF intraperitoneally, or by inhalation. In both *b* and *c*, doses given intraperitoneally were 100–200 mg per kg, whereas the inhalation dose consisted of a 1-h exposure of the animals to an atmosphere containing 0.23 mmol 3MF per l of air.

Finally, we should emphasise that in the present investigation on the toxicological properties of inhaled 3MF, we have administered rather high concentrations of the compound to facilitate the study of its biological effects within a very short period of exposure. In future toxicological evaluations, it will, of course, be important to study the effect of long-term exposure to concentrations of 3MF that are likely to occur in the environment. We further suggest that the potential toxicological consequences of ingestion of 3MF as a trace contaminant in the water supply should also receive attention. 3MF was originally detected in rainwater samples collected during an atmospheric smog alert¹.

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The cellular mode of action of the anti-epileptic drug 5, 5-diphenylhydantoin

THE molecular basis of the many effects of 5,5-diphenylhydantoin (DPH, Dilantin) remains obscure. In addition to being the drug of choice in the prevention of seizures¹, DPH is used to correct cardiac arrhythmias (mainly those caused by digitalis)², and to control myokymia (continuous muscle fibre activity)³, and excessive secretion of insulin⁴ or anti-diuretic hormone⁵. Side effects include hypotension, hyperglycaemia and disturbances of calcium homeostasis⁶. Among the actions proposed for DPH are stimulation of active sodium/potassium transport⁷, inhibition of passive sodium

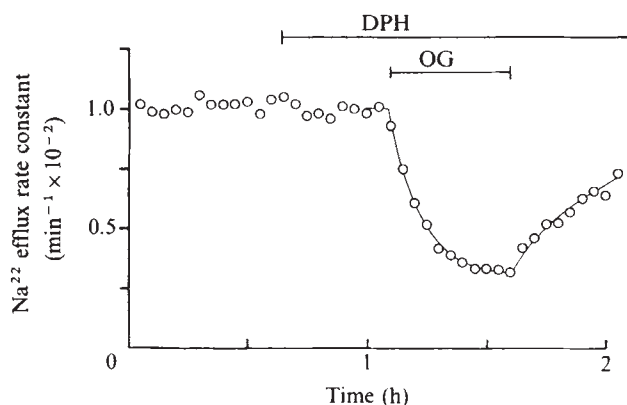
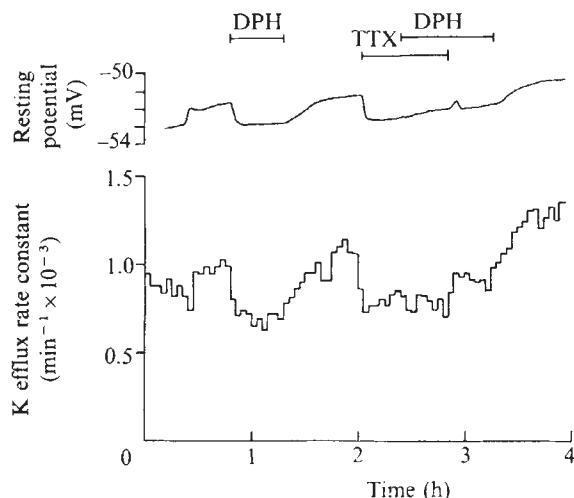


Fig. 1 Efflux of ^{22}Na from squid giant axon. The axon was exposed to flowing artificial seawater (ASW) of the following composition (in mM): NaCl, 425; KCl, 10; MgCl_2 , 25; MgSO_4 , 25; CaCl_2 , 10; Tris-HEPES (pH 7.8) 5; EDTA, 0.1. During the times indicated by the horizontal bars, the ASW contained 10^{-4} M 5,5-diphenylhydantoin (DPH) and 10^{-7} M ouabagenin (OG), respectively. Apparatus and technique were as described elsewhere¹³. Temperature 24 °C; axon diameter 410 μm .

influx in stimulated (but not in resting) cells^{8,9}, reduction of calcium influx^{10,11}, and specific interference with synaptic transmitter movements¹². We report here that DPH does not affect active sodium transport, but blocks passive resting sodium channels in much the same way as tetrodotoxin (TTX) does, and we suggest that from this elementary action may follow many of the drug's therapeutic, adverse, and *in vitro* effects.

Isolated giant axons of the squid, *Loligo pealei*, were micro-injected with ^{22}Na , whose active extrusion was subsequently monitored. DPH had no effect on sodium efflux (Fig. 1). Exposure of the axon to ouabagenin led to progressive sodium efflux inhibition, the rate of which was indistinguishable from that found in control experiments without DPH; recovery from cardiotoxic steroid poisoning was also unaffected. Further experiments (not shown) revealed no effect of DPH on binding

Fig. 2 Resting membrane potential of, and ^{42}K efflux from, squid giant axon. Artificial seawater (ASW) composition given in Fig. 1. At the time indicated by the arrow, 10^{-3} M ouabain was added to the ASW for the remainder of the experiment. (Ouabain is completely irreversible on the squid axon.) During the times indicated by the horizontal bars, the ASW also contained 2×10^{-4} M 5,5-diphenylhydantoin (DPH) or 10^{-7} M tetrodotoxin (TTX). Temperature 25 °C; axon diameter 500 μm . Membrane potential drifted about 2 mV over a 4-h period; the slow rise in baseline ^{42}K efflux is accounted for by this small depolarisation.



or release of ouabain, dihydro-ouabain, digitoxin, digitoxigenin monodigitoxoside, or digitoxigenin.

In a second series of experiments, axons were micro-injected with ^{42}K , and then monitored for ^{42}K efflux and resting potential. Figure 2 shows that DPH hyperpolarises the cell membrane much as TTX does¹⁴, that the effects of the two drugs are not additive, and occur even though the sodium pump is arrested, and that ^{42}K efflux follows the voltage variations, as expected from the dependence of potassium permeability on membrane potential¹⁵. DPH-induced membrane hyperpolarisation has also been reported recently for nodes of Ranvier¹⁶. Our findings eliminate stimulation of the electrogenic¹³ sodium pump or increased potassium permeability as mechanisms for the hyperpolarisation. Table 1 summarises the remainder of our measurements of resting membrane potential. Average hyperpolarisation caused by 10^{-4} M DPH was a little over half that produced by a supramaximal dose of TTX. The effects were never additive, and were abolished in Na-free seawater, as expected if DPH, like TTX¹⁷, blocked resting sodium channels. Voltage-clamp experiments on squid axon⁹ and Ranvier nodes^{16,18} have already established that DPH reduces the sodium current of action potentials.

Sodium channels of excitable cells can be 'held open' by compounds (for example, veratridine) which interfere with their inactivation mechanism¹⁹. If DPH blocks sodium channels in the manner of TTX, it should also reverse veratridine-induced membrane depolarisation and sodium influx, and this was found to occur (Fig. 3); these actions were not additive. In

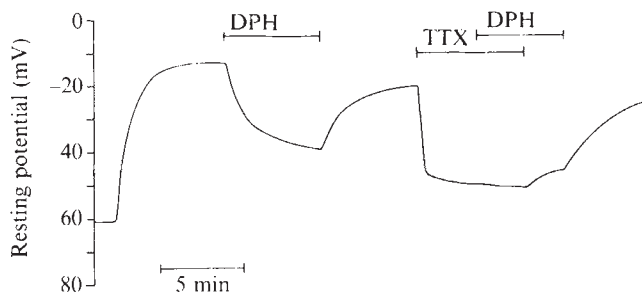


Fig. 3 Resting membrane potential changes induced in squid giant axon by veratridine, 5,5-diphenylhydantoin and tetrodotoxin. The axon was exposed to flowing artificial seawater of composition given in Fig. 1. At the time indicated by the arrow, 2.5×10^{-5} M veratridine was added for the remainder of the experiment. During the periods indicated by the bars, the seawater also contained 7.5×10^{-5} M 5,5-diphenylhydantoin (DPH) and/or 10^{-7} M tetrodotoxin (TTX).

sodium-free seawater (not shown) veratridine induced a small hyperpolarisation, that is, polarisation in the direction of the sodium equilibrium potential, which was reversed by DPH or TTX. We also examined the effect of DPH and TTX on veratridine-induced sodium influx into frog sartorius muscle. (Veratridine alkaloids are known to depolarise, and to increase sodium exchange in, frog muscle fibres²⁰.) Figure 4 shows that the extra sodium influx was 90% and 60% inhibited by TTX (10^{-7} M) and DPH (10^{-4} M), respectively.

From these and previous^{9,16,18} observations, we conclude that DPH, like TTX, blocks resting and excitable sodium channels, as well as those 'held open' by veratridine. The effects of the two drugs are not additive, and the resemblance between the urea moiety of DPH and the guanidine group of TTX raises the possibility that the molecules are indeed structural analogues. We also infer from our data that at least 50% of the TTX-sensitive sodium channels are blocked by DPH concentrations of the order of 10^{-4} M, and that, consequently, a clinical dose of 10^{-5} M might exert its therapeutic effect by blocking about 10% of the sodium channels.

Several effects of DPH are probably instantaneous consequences of its primary action on sodium permeability. For example, resting membrane hyperpolarisation and decreased

Table 1 Effect of tetrodotoxin (TTX) and 5,5-diphenylhydantoin (DPH), alone and in combination, on the resting membrane potential of squid giant axons bathed in sodium-containing or sodium-free artificial seawater

Measurement	Na seawater	Na-free seawater
Resting membrane potential	-57.5 ± 0.6 (22)	-54.8 ± 2.0 (8)
Change on addition of 10 ⁻⁷ M TTX	-1.8 ± 0.1 (17)	0.0 ± 0.1 (7)
Change on addition of 10 ⁻⁴ M DPH	-1.0 ± 0.1 (14)	0.0 ± 0.2 (6)
Change on addition of both DPH and TTX	-1.5 ± 0.2 (9)	0.0 ± 0.1 (5)

All values are means ± s.e.m., in mV; the sign is that of the cell interior. The number of observations is given in parenthesis. Seawater composition is given in the legend of Fig. 1. Temperature 23 ± 2° C.

sodium conductance must raise the firing threshold of, and so 'stabilise', excitable membranes. Such 'stabilisation' could possibly be enhanced by the reduction of potassium efflux to the pericellular space (Fig. 2). Hyperpolarisation, in addition to reduced intracellular calcium (see below), will also interfere with stimulus-secretion coupling; thus, DPH hyperpolarises pancreatic islet cells²¹ and inhibits insulin release⁴, as does TTX²². It is likely that similar mechanisms are involved in the lowering of antidiuretic hormone²³, glucagon²⁴, calcitonin²⁵ and catecholamine²⁶ secretion by DPH. A second class of DPH effects may be viewed as mediated by the steepened sodium electrochemical gradient which will result from prolonged inhibition of passive sodium influx. The sodium gradient is the energy source for cellular uptake of many organic molecules, including transmitters²⁷, and is also a major regulator of intracellular calcium levels ([Ca]_i)²⁸. Consequently, if the inotropic and toxic effects of digitalis result from sodium pump inhibition (through reduction of the sodium gradient and increased [Ca]_i), inhibition of passive sodium permeability by DPH will restore the gradient and correct the anomalies. A lowering of [Ca]_i in vascular smooth muscle will produce hypotension²⁸. Another effect of DPH is to attenuate²⁹ post-tetanic potentiation, which presumably results from calcium accumulation in presynaptic nerve terminals³⁰; here again, limitation of sodium influx (in addition to limitation of calcium influx through the sodium channel) may steepen the sodium gradient sufficiently to prevent excessive presynaptic calcium accumulation. Since [Ca]_i affects transmitter release, and sodium gradients energise transmitter reuptake, extraordinarily complex effects of DPH on transmitter movements should be anticipated. Finally, such consequences of depolarisation as elevated cyclic nucleotide levels³¹

will be prevented by DPH, provided that they can be traced to the opening of sodium channels or the inhibition of sodium pumping³².

Thus, it seems that many actions of 5,5-diphenylhydantoin can be ascribed to a single demonstrable primary event: the specific inhibition of resting sodium permeability. Whether the drug has additional primary actions (such as the blocking of calcium channels) remains to be determined.

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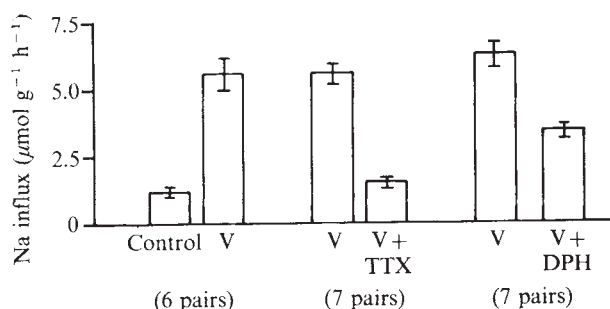


Fig. 4 Effect of veratridine, tetrodotoxin, and 5,5-diphenylhydantoin on sodium influx into *Rana pipiens* sartorius muscles. Freshly excised muscle pairs were depolarised to about -30 mV by placing them in a high-potassium medium, in order to minimise the difficulty of comparing fluxes in cells of widely differing resting potentials (veratridine causes depolarisation in muscle¹⁹). The bathing fluid contained (in mM): Na⁺, 43; K⁺, 43; Ca²⁺, 2; Cl⁻, 10; SO₄²⁻, 39; sucrose 103; phosphate buffer, 1.6 (pH 7.1), and ouabain, 10⁻⁴ M. Some solutions contained veratridine (V) (10⁻⁴ M), tetrodotoxin (TTX) (10⁻⁷ M), and/or 5,5-diphenylhydantoin (DPH) (10⁻⁴ M), as indicated. After a 10-min preincubation period, muscles were exposed for 20 min to similar solutions containing ²²Na; total intracellular radioactivity was then determined by back extrapolation of the linear portion of a 90-min semi-log washout curve. Bar heights are means ± s.e.m. Correction for efflux during the influx period amounted to less than 5% in each case, and was therefore neglected.

Actin-related gelation of Ehrlich tumour cell extracts is reversibly inhibited by low concentrations of Ca²⁺

THE intracellular filament system has been shown to have an important role in several biological phenomena¹⁻³, but the mechanisms of control of the organisation of these filaments are little understood. Temperature-induced gela-

Table 1 Inhibition of gelation by Ca^{2+} and its reversal by EGTA

Total Ca^{2+} (mM)	Calculated free Ca^{2+} (μM)	Gelation Incubation time (min)					
		10	20	35	45	60	75
0	0	++	+++	+++		+++	+++
0.5	0.78	+	++		++	++	
0.6	1.17	—	—	—		—	—
0.8	2.95→0.06	*—	+++	+++		+++	+++
0.8	2.95→0.06	—	—	—*		+++	+++

Cell-free extract was filtered through a Sephadex G-25 column equilibrated with the extraction medium to remove endogenous Ca^{2+} and the eluate containing 7.6 mg of protein in 0.4 ml were used. Gelation was performed at pH 7.0. Degree of gelation and calculation of free Ca^{2+} concentrations were performed as described in the legend to Fig. 2. *EGTA in a volume of 20 μl was added to a final concentration of 10 mM (free Ca^{2+} concentration falling to 0.06 μM , as shown).

tion of cell-free extracts⁶⁻¹¹ provides a convenient system for studying interaction of cytoskeletal components in cell-free conditions. Actin and a high molecular weight (MW) actin-binding protein (MW 270,000) (ABP) are concentrated in the gel formed by warming of mammalian cell extracts⁹. In other cell types, protein other than actin and ABP are also required for gelation, such as 58,000 MW protein in sea urchin egg extracts¹⁰ and four low molecular weight proteins (23,000 to 38,000) for amoeba extracts¹¹. A requirement of ATP for gelation⁶ and inhibition of the reaction by cytochalasin B^{8,9} have been found recently. We now describe reversible inhibition of gelation by micromolar concentration of Ca^{2+} and possible involvement of this type of regulation in a virus-induced cell fusion.

Ehrlich tumour cells were suspended in equal volumes of extraction medium containing 0.34 M sucrose, 1 mM ATP, 1 mM dithiothreitol, 40 mM Tris-maleate (pH 7.0) and 1 mM EGTA. Cells were disrupted by releasing the cell suspension from a nitrogen pressure cell in which they were equilibrated with nitrogen gas at 1,300 to 1,500 lb inch⁻². Cell-free extracts obtained after ultracentrifugation of the cell lysates formed a gel when MgCl_2 was added to a final concentration of 2.5 mM and then incubated at 20 °C. If free Ca^{2+} concentrations in the reaction mixture were adjusted to micromolar concentrations with EGTA- Ca^{2+} buffer system¹², gelation was inhibited. Addition of excess EGTA to the extracts, which were preincubated with inhibitory concentrations of Ca^{2+} at 20 °C for 10 or 35 min, induced gelation as shown in Table 1 and Fig. 2. Thus, inhibition by high concentrations of free Ca^{2+} was reversible. Inhibition of gelation by micromolar concentrations of Ca^{2+} was observed in sea urchin egg extracts by Kane¹⁰. Analysis of the composition of the gel (Fig. 1) showed that a protein with the same mobility as actin, and a high molecular weight protein corresponding to actin binding protein of Hartwig and Stossel¹³ are concentrated in the gel. Myosin is also present in the gel but not actually concentrated in it from the extracts. The amounts of total protein (data not shown), 'actin' and 'ABP' in the pellets of low speed centrifugation, that is, in the gel, are decreased by increasing free Ca^{2+} concentrations (Fig. 2). Examination of 'ABP' to 'actin' ratios in the pellets obtained at different Ca^{2+} concentrations, shows that 'actin' is less concentrated in the pellet than 'ABP' at high Ca^{2+} concentrations. In contrast, in the gel formed at a partially inhibitory concentration of cytochalasin D (0.1 $\mu\text{g ml}^{-1}$), 'ABP' to 'actin' ratio was lower than that of the control gel. Thus, Ca^{2+} and cytochalasin D, although both inhibitory, seem to have distinctly different effects on gel formation.

Although the 'actin' present in the gel has a molecular weight similar to that of authentic actin, experiments

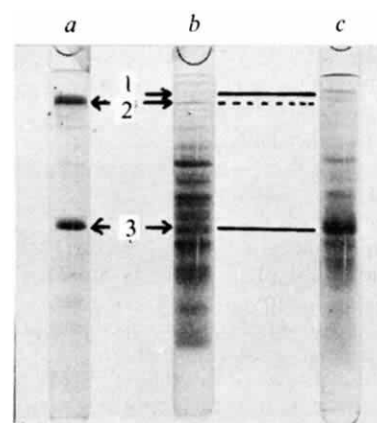
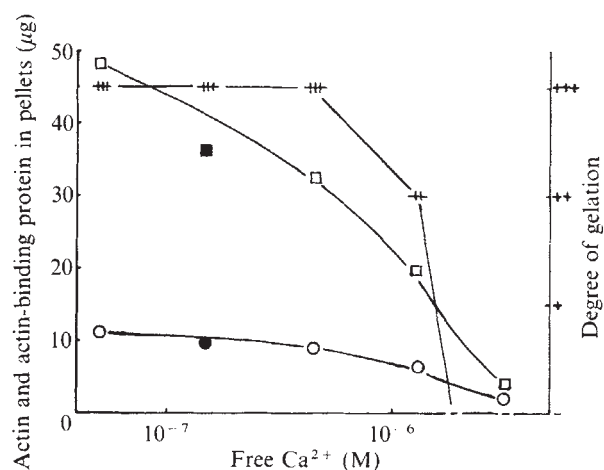


Fig. 1 SDS-disc electrophoresis of cell free extracts and gels *a*, Control; myosin and actin of rabbit skeletal muscle. *b*, Ehrlich tumour cell extracts. *c*, Gel formed after incubation at 25 °C for 60 min at a free Ca^{2+} concentration of 3.1 μM was pelleted at 2,000g for 30 min. The pellet was used for electrophoresis. Bands marked 1 (actin-binding protein) and 3 (actin) were concentrated in the gel. Band 2, myosin heavy chain.

confirming this identification may be required. Evidence to support the idea of the involvement of actin in the gelation comes from the presence of branched bundles of thin filaments in negatively-stained gels (Fig. 3). This structure was not observed in extracts in which gelation was inhibited by the addition of Ca^{2+} (data not shown). Furthermore, gelation was almost completely inhibited by the addition of DNase I (Sigma type DN-EP; 0.1 mg per 2.5 mg protein of

Fig. 2 Inhibition of gelation with Ca^{2+} . The degree of gelation is expressed as follows; + + +, firm gel which could turnover easily without breaking the gel; + +, gel which showed no appreciable deformation when the tube containing it was brought to horizontal position; +, gel which deformed but could resist tilting of the tube; —, no gelation or gel easily broken down with slight disturbance. Extracts which were subjected to gel filtration as described in the legend of Table 1 were used. A CaCl_2 solution prepared from CaCO_3 was added and free Ca^{2+} concentrations were calculated according to Ogawa¹². Final pH after gelation was 6.7. The eluate containing 8.17 mg protein in 0.4 ml was used in each tube, and pellets were washed two times with 0.1 M KCl. Open symbols, amounts of actin (□) and actin-binding protein (○) in the pellets. Calculation was based on the assumption that all protein was stained equally by Coomassie brilliant blue and amounts were expressed as μg protein. Solid symbols, amounts of actin (■) and actin-binding protein (●) in a sample brought to a free Ca^{2+} concentration of 3.1 μM , incubated for 30 min at 20 °C and then brought back to 0.15 μM by the addition of EGTA. Duration of incubation for gelation at 20 °C was 60 min.



the extract) which is known to bind rather specifically to actin¹⁴. Preincubation of DNase I with 1.5 times (by weight) of muscle actin effectively abolished the inhibition (data not shown; similar independent observations have been made by M. Ishiura and Y. Okada). Preformed gels were dissolved by layering DNase I solution on top of the gel. Thus, participation of actin in the gelation seems to be substantiated.

If formation and dissolution cycle of actin-related gel plays an important part in cell locomotion, phagocytosis and other biological phenomena as Stossel suggested¹⁵, and if Ca^{2+} has a regulatory role in the gelation as postulated here, then Ca^{2+} should dissolve preformed gels. As is shown in Fig. 4a, partial dissolution of the preformed gel was attained within a few minutes by layering Ca^{2+} -containing medium on top of the preformed gel; prolonged incubation or gentle mixing of the medium resulted in further dissolution of the gel.

Further evidence for the involvement of Ca^{2+} regulation in the cytoskeletal system was obtained from the studies on virus-induced cell fusion. As previously reported by Okada¹⁶, extracellular Ca^{2+} was required for maximal fusion of Ehrlich tumour cells induced by HVJ (Sendai virus). Furthermore, cyclic AMP-dependent stimulation of the fusion reaction is also maximal at an extracellular Ca^{2+} concentration of 0.5 mM (ref. 17). Mere presence of extracellular Ca^{2+} is, however, not enough for stimulation of fusion in the latter case, since addition of a Ca^{2+} permeation inhibitor, ruthenium red, abolished the effect of Ca^{2+} on the stimulation induced by increases of intracellular cyclic AMP¹⁸. The gel-forming ability of cell-free extracts prepared during Sendai virus-induced fusion was therefore examined. Cells were incubated in a fusion medium¹⁷ containing 1 mM Ca^{2+} with or without virus at 37 °C for 15 min and then pelleted. Cell-free extracts were prepared from the

Fig. 3 Negative staining of the gel. Extracts prepared in standard medium were incubated at 20 °C for 30 min. Gel formed in this way was transferred with minimum disturbance on a grid and stained with 1% phosphotungstate (pH 7.0), then observed by a Hitachi HU-12 electron microscope in Y. Tashiro's laboratory of Kansai Medical University. Magnification $\times 50,000$.

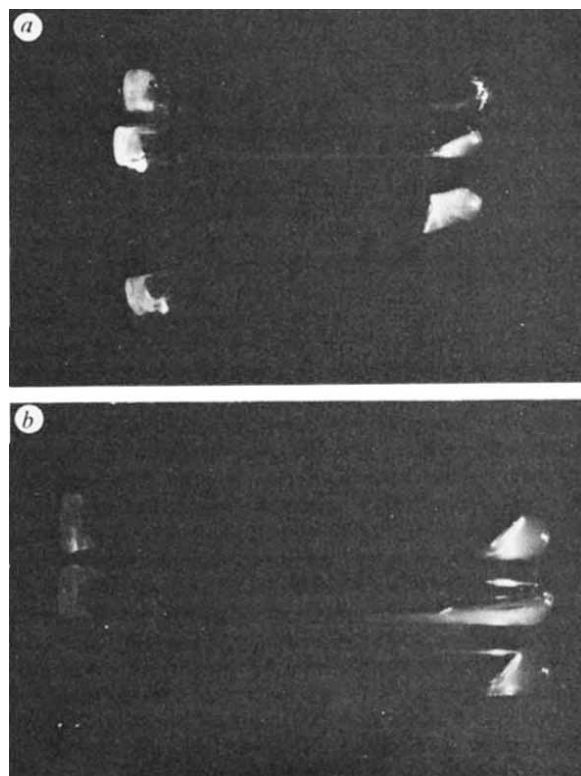


Fig. 4 Dissolution of the gel by Ca^{2+} and inhibition of gelation by Sendai virus-induced cell fusion. *a*, Top tube, Ca^{2+} was added to a final concentration of 0.8 mM. Middle tube, preformed gel was partially dissolved by the addition of Ca^{2+} to a final concentration of 0.8 mM (final free Ca^{2+} concentration calculated to be more than 3.1 μM). Duration of incubation with Ca^{2+} was 30 min without disturbance. Bottom tube, gel formed by incubation of untreated extracts at 20 °C for 150 min. Total protein contents were 10 mg in 0.4 ml. *b*, Top tube, the same extract as below but with EGTA added to a final concentration of 10 mM. Middle tube, cell-free extract obtained from 1.84×10^9 cells suspended in 80 ml medium and fused with 2,500 haemagglutinating units per ml of virus in highly aerobic conditions for 15 min at 37 °C. No gelation was observed after incubation for 240 min at 25 °C. Bottom tube, gel of ++ degree, extract from untreated control cells. EGTA was added to a final concentration of 0.15 mM to middle and bottom tubes.

pellets with the medium containing 2.0 mM EGTA (final concentration, 1.2 mM). Extracts prepared in this way either from control or fusing cells have the ability to gel. Back titration of the extracts with Ca^{2+} revealed differences in the properties of these extracts. Addition of free Ca^{2+} to 0.8 $\mu\text{mol ml}^{-1}$ completely inhibited the gelation of extracts derived from fusing cells, whereas the same amount of Ca^{2+} had no effect on the gel formation of extracts of control cells. A Ca^{2+} concentration of over 1.0 $\mu\text{mol ml}^{-1}$ was required for the inhibition of gelation in control cell extracts.

In other experiments, extracellular Ca^{2+} was removed by washing the pellets with sodium citrate-saline twice, and then washed pellets were disrupted at a low EGTA concentration (0.05 mM). Extracts prepared in this way from untreated cells gelled at 0.1 mM EGTA, whereas no gelation was observed with extracts of fused cells by EGTA at up to 0.25 mM (Fig. 4b); removal of free Ca^{2+} from the latter extracts either by gel filtration through Sephadex G-25 or by further addition of EGTA to a final concentration of 10 mM restored gel forming ability. Thus, the intracellular Ca^{2+} concentration seems to increase during virus-induced cell fusion, and this increased Ca^{2+} , in turn, inhibits gel formation. Although a possible involvement of microfilaments in virus-induced cell fusion has already been

suggested by us⁵, the recognition of reversible inhibition of actin-related gelation by intracellular Ca^{2+} described above may clarify the possible mode of control of intracellular organisation of microfilaments during the fusion process. It should be noted that a low concentration of cytochalasin D ($0.5 \mu\text{g ml}^{-1}$) was found to inhibit both gelation and virus-induced fusion almost completely⁵. More complex regulation, however, could be operating in the virus-treated cells.

Stossel and Hartwig⁷ reported that gelation of a reconstituted system composed of actin, ABP, myosin and a cofactor protein purified from macrophage was not appreciably inhibited by 1.3 mM CaCl_2 in the absence of EGTA. Therefore, the inhibition of gelation in crude extracts by low concentrations of Ca^{2+} is probably explained by the presence of a Ca^{2+} -sensitivity conferring factor(s) in the crude extracts. Experiments to test this idea are in progress.

After submission of this letter, we became aware of a paper by Condeelis and Taylor¹⁹ describing inhibition of gel-sol transformation of *Dictyostelium* extracts by low concentrations of Ca^{2+} and its possible significance in regulation of motility. Since our results are in accordance with theirs, this type of regulation seems to have an ubiquitous role in a wide variety of organisms.

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Presynaptic muscarinic cholinergic receptors

In the isolated rabbit heart, acetylcholine inhibits the release of noradrenaline evoked by nicotinic drugs¹, by KCl (ref. 2) and by sympathetic nerve stimulation³. The release of noradrenaline in response to sympathetic nerve stimulation in the rabbit heart was found to be reduced by parasympathomimetic agonists, and atropine antagonised this effect of cholinergic drugs. Löffelholz and Muscholl² therefore suggested that the inhibitory effect of acetylcholine on catecholamine release is mediated by the interaction of acetylcholine with the muscarinic cholinergic receptors located at the terminals of sympathetic nerve fibres. Several workers, using the perfused rat mesenteric arteries and the perfused rabbit ear artery, demonstrated that low concentrations of acetylcholine inhibit the vasoconstrictor response to sympathetic nerve stimulation⁴⁻⁶, and atropine also antagonises the inhibitory effects of these cholinergic drugs in a competitive manner⁷. Thus, there is considerable indirect physiological evidence for the existence of muscarinic

cholinergic receptors in the sympathetic nerve endings. In this report, we provide direct biochemical evidence suggesting the presence of muscarinic cholinergic receptors in the peripheral catecholaminergic nerve endings in the rat myocardium.

The sympathetic nerve endings of peripheral organs may be selectively destroyed by the intravenous administration of 6-hydroxydopamine^{8,9}. A predominant localisation of [³H]-ouabain binding sites in the sympathetic nerve endings of cat heart was demonstrated by measuring specific [³H]-ouabain binding to cardiac microsomal ($\text{Na}^+ + \text{K}^+$)-ATPase obtained from cats which had been previously treated with saline or 6-hydroxydopamine^{10,11}. A similar approach was adopted to determine possible localisation of muscarinic cholinergic receptors in the sympathetic nerve terminals of rat heart by using a potent muscarinic antagonist [³H]-quinuclidinyl benzilate (QNB), which has been shown to bind selectively to muscarinic receptors in the microsomal fractions from brain¹², intestine¹³ and heart¹⁴.

Specific [³H]-QNB binding to the particulate fractions obtained from heart in control and in 6-hydroxydopamine treated rats is shown in Table 1. When the concentration of [³H]-QNB was 0.25 nM , the specific binding to the control preparation was found to be 908 c.p.m. per mg protein. Administration of 6-hydroxydopamine decreased the specific binding of [³H]-QNB to muscarinic cholinergic receptors to about 70% of control (Fig. 1). This decrease in specific binding to the cardiac microsomal fraction may be due to the alteration in the apparent affinity of these binding sites to QNB or to a decrease in the number of muscarinic cholinergic receptor sites. The dissociation constants and the densities of the muscarinic cholinergic receptors in control and 6-hydroxydopamine treated rat heart particulate fractions were determined by measuring the specific binding with various concentrations of [³H]-QNB, and analysing the data by the method of Scatchard¹⁵ (Fig. 1). The dissociation constant of [³H]-QNB binding to control rat heart particulate fraction was found to be 0.46 nM , and this value is similar to the affinity constant of muscarinic cholinergic receptors to QNB in 6-hydroxydopamine treated rat heart preparations (0.40 nM). Although the dissociation constant of [³H]-QNB to muscarinic cholinergic receptors did not increase significantly, the density of receptors

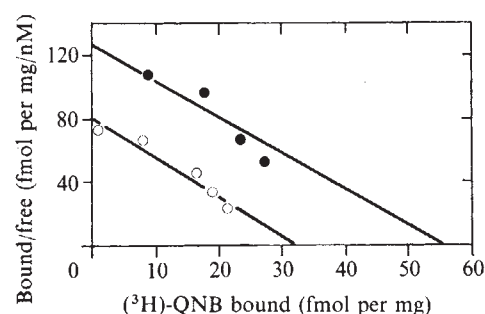


Fig. 1 Scatchard plot of [³H]-QNB binding to cardiac particulate fractions from sympathectomised and control rats. Cardiac particulate fractions were incubated with a series of concentrations of [³H]-QNB, and specific binding was determined as described in Table 1. The lines for control ($r = 0.91$) and sympathectomised ($r = 0.88$) rat heart particulate fractions were determined by linear regression analysis. Each point represents the mean of triplicate determinations. The negative reciprocal of the slope provides an estimate of the equilibrium dissociation constant (K_D) for the interaction of [³H]-QNB with the muscarinic cholinergic receptors, and the values of K_D for control and sympathectomised rat heart preparations were found to be 0.46 nM and 0.40 nM , respectively. The maximal number of binding sites (B_{max} for control (●) and sympathectomised (○) rat cardiac microsomes) was found to be 56 and 32 fmol per mg protein, respectively.

Table 1 Effect of chemical sympathectomy or chronic reserpine treatment on specific binding of [³H]-QNB to heart particulate fractions

Drug treatment	Concentration of [³ H]-QNB (nM)	Total counts (c.p.m. per mg protein)	Binding of [³ H] QNB	
			Nonspecific accumulation (c.p.m. per mg protein)	Specific binding (c.p.m. per mg protein)
None	0.05	271 ± 8.21	105 ± 7.22	166 ± 8.19
6-Hydroxydopamine	0.05	208 ± 4.12	135 ± 8.98	73* ± 5.26
None	0.25	1,291 ± 63.29	383 ± 12.19	908 ± 50.46
6-Hydroxydopamine	0.25	854 ± 39.49	220 ± 8.12	634* ± 56.49
None	0.05	271 ± 8.21	105 ± 7.22	166 ± 8.19
Reserpine	0.05	298 ± 6.16	116 ± 5.11	182 ± 7.56
None	0.25	1,291 ± 63.29	383 ± 12.19	908 ± 50.46
Reserpine	0.25	1,034 ± 54.68	212 ± 11.56	822 ± 41.53

Male Sprague-Dawley rats (120–160 g) were divided into four groups of 10 rats each. To destroy the catecholaminergic nerve endings, the rats of the first group were treated with intravenous injections of 6-hydroxydopamine hydrobromide, two doses of 50 mg per kg body weight on the first day and two doses of 68 mg per kg one week later⁸. These animals were killed two weeks after the first dose of 6-hydroxydopamine. The Na⁺-dependent [³H]-noradrenaline uptake into the slices of the spleens of these rats was found to be inhibited by >90% as compared to the uptake into the spleen slices from control rats. The second group of rats, which served as control, received equal volumes of saline. The rats of the third group received daily intraperitoneal injections of reserpine (0.5 mg per kg) for 7 d and were killed on the morning of the eighth day. The fourth group of rats, which served as control, received equal volumes of saline. [³H]-QNB (29.4 Ci per mmol) was purchased from New England Nuclear, and purity of the compound was tested by thin-layer chromatography¹²; its specific activity was 38.62 c.p.m. per fmol. The procedure for the preparation of heart particulate fractions has been described¹⁴, and was similar to that adopted for rat brain¹⁵. The specific binding of [³H]-QNB was measured by the method of Yamamura and Snyder¹² and has been described elsewhere¹⁴. Corrections were made for nonspecific accumulation of radioactivity by assaying parallel incubations which contained a large excess (100 µM) of oxotremorine. The results shown are the means ± s.e.m.

* Significantly different from control ($P < 0.001$).

markedly decreased in cardiac microsomes, from 56 fmol per mg protein to 32 fmol per mg protein, following chemical sympathectomy. This would suggest that chemical sympathectomy leads to a decrease in the density of muscarinic cholinergic receptors in rat cardiac particulate fractions.

Chemical sympathectomy may decrease muscarinic cholinergic receptor density in cardiac microsomal fractions by at least two mechanisms. Because the administration of 6-hydroxydopamine leads to degeneration of sympathetic nerve terminals, the consequent decrease in the density of muscarinic cholinergic receptors in heart particulate fractions may be due to loss of muscarinic cholinergic receptors located at the presynaptic sites on the noradrenergic neurones. Again, ablation of the motor nerve increases the number of β -adrenergic receptors in the gastrocnemius muscle particulate fraction¹⁷. Thus, another possible mechanism by which chemical sympathectomy might decrease the specific binding of [³H]-QNB would be the loss of regulation of postjunctional muscarinic cholinergic receptors by noradrenergic neurones.

The latter possibility was tested by measuring specific binding of [³H]-QNB to muscarinic cholinergic receptors of cardiac particulate fractions obtained from rats which had been chronically treated with reserpine. Reserpine destroys the storage mechanisms for catecholamines and thus terminates sympathetic transmission without the degeneration of noradrenergic nerve terminals. Thus, if sympathetic transmission is involved in the regulation of postjunctional muscarinic cholinergic receptors, then chronic reserpine treatment would lead to a decrease in specific binding of [³H]-QNB to cardiac particulate fractions. On the other hand, if some muscarinic cholinergic receptors in control heart preparations are located at the presynaptic sites in the noradrenergic nerve terminal, then chronic treatment with reserpine should not alter specific binding of [³H]-QNB to heart preparations. Results shown in Table 1 indicate that chronic treatment with reserpine does not modify specific binding of [³H]-QNB to heart particulate fractions, suggesting that the decrease seen in the specific binding of QNB following chemical sympathectomy is due to loss of presynaptic muscarinic cholinergic receptors located in the noradrenergic neurones.

Finally, Fozard and Muscholl¹⁸ examined the possibility that the properties of prejunctional muscarinic cholinergic

receptors may be different from those located in postjunctional sites, by comparing the relative potencies of a series of muscarinic agonists for their ability to decrease the electrically induced release of noradrenaline (prejunctional effect) from perfused rabbit heart on the one hand, and their ability to inhibit atrial tension development and ventricular rate (postjunctional effects) on the other hand. These investigators observed that the muscarinic

Table 2 Relative potencies of drugs in reducing specific binding of [³H]-QNB to control and chemically sympathectomised rat heart particulate fractions

Drug	ED ₅₀ * (M)	
	Control	6-hydroxydopamine treated
Atropine	8×10^{-10}	8.5×10^{-9}
Scopolamine	9.5×10^{-10}	5.7×10^{-9}
Carbamylcholine	2.2×10^{-7}	7.2×10^{-6}
Pilocarpine	3.4×10^{-7}	6.5×10^{-6}

Values are mean data from three separate experiments performed in triplicate, whose results varied by less than 15%.

*Concentration of drug which displaced specific binding of [³H]-QNB by 50%.

potency of the compounds was similar in each of the three systems examined¹⁸. Also, apparent affinities of prejunctional and postjunctional muscarinic cholinergic receptors seem to be the same in perfused rabbit ear artery or guinea pig vas deferens¹⁹. Despite such observations, there is a small but detectable difference in the ability of some cholinergic agonists and antagonists to reduce specific binding of [³H]-QNB by 50% in control and chemically sympathectomised rat heart particulate fractions, suggesting that the affinity constants of prejunctional and postjunctional muscarinic receptors for cholinergic drugs may be different (Table 2). It is possible that differences in physiological responses with different affinity constants may have prevented previous workers^{7,18,19} detecting small changes in apparent affinity of these two populations of receptors; these may be revealed by measuring the initial step of drug-receptor interaction.

Thus, we conclude that a substantial fraction of muscarinic cholinergic receptors in cardiac particulate fractions is located at the presynaptic or prejunctional sites in the sympathetic nerve terminals innervating the myocardium, and these presynaptically located muscarinic cholinergic receptors may play an important part in the regulation of the secretion of catecholaminergic nerve endings.

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Effects of lisuride and LSD on cerebral monoamine systems and hallucinosis

THE levels and turnover of 5-hydroxytryptamine (5-HT), noradrenaline (NA) and dopamine (DA) suggest that monoaminergic neurones (particularly serotonergic ones) and their postsynaptic receptors are the main targets of LSD action in man and animals¹⁻⁴. Because this evidence still seems inconclusive⁴, we have compared here the effects of LSD with those of lisuride on the biochemistry of brain amines in the rat. Lisuride is an ergot derivative recently introduced for the treatment of migraine, and in oral doses of up to 600 µg, is without hallucinogenic properties in man⁵. This lack of psychotomimetic effect is unlikely to be due to poor bioavailability after oral administration, because, at least in the rat, oral lisuride was even more effective than after intraperitoneal injection in altering monoamine turnover⁶. In addition, we have studied the effects of LSD and lisuride after intracerebral injection into the raphe nuclei. The present findings do not support the hypothesis that the hallucinogenic effect of LSD is reflected by variations in the level and turnover of the brain amines. In particular, a stimulation of 5-HT autoreceptors is also evident after the non-hallucinogenic lisuride.

The experiments were carried out on male albino rats of Wistar origin (stock Füllinsdorf, SPF, 150-200 g). Brain amines and metabolites were determined spectrofluorimetrically⁶, and adenylate cyclase activity was measured in striatal homo-

Table 1 Effects of lisuride and LSD on the levels of monoamines and their metabolites in rat whole brain

	% Cerebral levels (control = 100)	
	Lisuride	LSD
5-HT	110.5 ± 2.3 (13)*	107.4 ± 2.3 (13) NS
DA	113.3 ± 2.2 (13)†	110.0 ± 2.7 (13)†
NA	71.7 ± 3.1 (13)†	91.1 ± 2.8 (13)‡
5-HIAA	81.5 ± 2.5 (9)†	89.2 ± 2.7 (16)‡
HVA	64.5 ± 4.8 (9)‡	80.3 ± 3.9 (16)*
MOPEG-SO ₄	181.0 ± 10.6 (8)‡	119.5 ± 4.5 (20)†

Lisuride or LSD was injected intraperitoneally at a dose of 1 mg per kg (for amines and MOPEG-SO₄) or 0.1 mg per kg (HVA, 5-HIAA), and the rats were decapitated 30 min (amines) or 60 min (metabolites) after drug injection. The tissue levels are given as the % of those found in saline-injected controls and are means ± s.e.m.; number of determinations (1 rat each) in parentheses. Statistical significance for difference from control value (*t*-test): * *P* < 0.05; † *P* < 0.001; ‡ *P* < 0.01; others *P* > 0.05. Absolute control values (ng per g wet tissue, means ± s.e.m. of 12-16 single values): 5-HT, 692 ± 38; DA, 733 ± 8; NA, 422 ± 10; 5-HIAA, 349 ± 11; HVA, 70 ± 5; MOPEG-SO₄, 144 ± 9. A more detailed biochemical study in discrete brain areas (cortex, limbic forebrain, striatum and hypothalamus) yield virtually the same results¹².

genates⁷ and cyclic AMP formation in limbic forebrain slices⁸. Unless otherwise stated, all compounds were injected intraperitoneally, and the doses refer to the salts, the hydrogen maleate of lisuride and the tartrate of LSD. In some animals, lisuride or LSD was injected stereotactically into the brain.

In rats injected with lisuride (1 mg per kg body weight) 30 min before decapitation, there was a slight increase in brain levels of 5-HT and DA and a decrease in cerebral NA to about 72% of the control value (Table 1). LSD injection produced similar changes (Table 1 and ref. 1). In addition, 0.1 mg per kg of lisuride induced, within 1 h, a significant decrease of 5-hydroxyindolacetic acid (5-HIAA) level and a marked reduction (to 65%) of homovanillic acid (HVA, Table 1) level. In contrast, 3-methoxy-4-hydroxyphenylethyleneglycol sulphate (MOPEG-SO₄) concentration was increased, the maximal elevation (181%) being reached at a dose of 1 mg per kg (Table 1). These findings agree with recent evidence indicating that the accumulation of L-DOPA after decarboxylase inhibition is enhanced by lisuride in NA-rich brain regions, but is decreased in DA-rich areas⁹.

Table 1 also shows that lisuride produces biochemical changes similar to, but much more marked than, those reported to occur after LSD¹⁻¹⁰. The greater potency of lisuride is particularly apparent with respect to the DA system, as the reduction in the cerebral HVA level was more pronounced and longer lasting than that seen with LSD¹¹⁻¹⁶.

Both lisuride and LSD cause a similar decrease in 5-HT turnover, as judged by the reduction of the cerebral 5-HIAA levels. In order to see whether lisuride has a direct effect on 5-HT neurones similar to that proposed for LSD on the basis of microiontophoretic experiments¹⁷, both drugs were injected stereotactically into a brain area close to the raphe nuclei. Lisuride, but not LSD, caused a reduction of the 5-HIAA level and antagonised the acceleration of the 5-HT turnover induced by methiothepin, a potent 5-HT receptor-blocking agent¹⁸ (Fig. 1).

These findings suggest that lisuride preferentially stimulates receptors located on the perikaryal membrane of 5-HT neurones (somatic autoreceptors), a view further strengthened by the observation that the intense mounting behaviour elicited by lisuride in male and female rats, presumably due to removal of serotonergic inhibition¹⁹, can be counteracted by compounds which stimulate postsynaptic 5-HT receptor sites, such as 5-hydroxytryptophan or quipazine. The lack of effect of even large amounts of LSD (20 µg per rat) injected stereotactically cannot be explained by its short half life in the CNS²⁰, since even six repeated intraperitoneal injections of LSD (1 mg

per kg), at 30 min intervals, did not further decrease brain 5-HIAA levels significantly⁶. Furthermore, although it has been shown that single raphe neurones stop firing after much smaller doses of LSD (3 µg per kg intravenously)²¹ than those used by us (1 mg per kg intraperitoneally, six injections), it may be postulated, on the basis of our biochemical results, that the firing rate of only a minor fraction of the whole neuronal population of the raphe nuclei is inhibited by LSD. A sampling bias possibly underlying the recording criteria for single raphe units may account for the discrepancy between the electrophysiological results and the present data. Alternatively, a dissociation may exist between firing rate and 5-HT turnover of the raphe neurones, that is, a reduction of the spiking activity without a proportional diminution in 5-HT turnover.

In analogy to other ergot derivatives such as lergotril, 2-bromo- α -ergocryptine and ergometrine²², and in contrast to LSD¹⁰, lisuride did not stimulate *in vitro* cyclic AMP formation in striatal homogenates, even at concentrations of 10^{-4} M (Fig. 2). This could indicate that metabolites of these drugs may be the active agents. In contrast, lisuride inhibited very potently (IC_{50} , 1.2×10^{-7} M, see Fig. 2) the DA-stimulated formation of cyclic AMP *in vitro*, whereas LSD was much weaker in this respect (IC_{50} , 1×10^{-5} M)¹⁰. The formation of cyclic AMP in slices of the limbic forebrain stimulated by NA (1×10^{-5} M) was more effectively antagonised by lisuride (IC_{50} , 8×10^{-8} M) than by LSD (IC_{50} , 3×10^{-6} M)^{6,11,12}. This observation, as well as the increase in MOPEG-SO₄ which was also more pronounced after lisuride than after LSD, suggest that lisuride is a more potent blocker of NA receptors than LSD.

Our findings therefore challenge the generally accepted

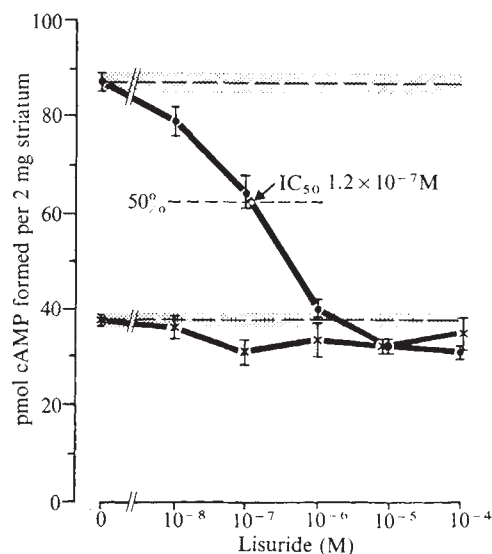
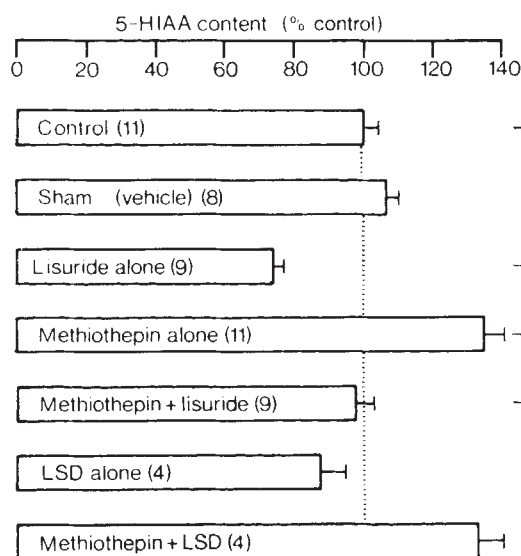


Fig. 2 Effect of lisuride at various concentrations applied either alone (x) or in combination with 3×10^{-6} M dopamine (●) on adenylate cyclase activity in homogenates of rat striatum measured essentially as described elsewhere⁷. Each point is a mean with s.e.m. of at least four determinations in duplicate.

Fig. 1 5-HIAA levels in rat forebrain after infusion of lisuride or LSD into the medial and dorsal raphe nuclei. Rats with or without pretreatment with methiothepin maleate (10 mg per kg injected intraperitoneally 15 min earlier) received local infusion of either lisuride or D-LSD under halothane anaesthesia (halothane, 3.5 vol % for induction (5 min), 1% for maintenance, in air enriched with oxygen to 50%, start of anaesthesia 10 min before infusion). The ergot derivatives were infused at a rate of $1 \mu\text{l min}^{-1}$ by means of a cannula (diameter 0.3 mm) with its tip placed stereotactically in an area immediately adjacent to the raphe medianus (mr) or dorsalis (dr) using the following coordinates (König and Klippel³¹): mr, A, 0.4, L, + and - 0.8, V, 2.9; dr, A, + 0.4, L, + and - 0.8, V, - 0.6. To both the mr and the dr, one infusion ($5 \mu\text{g } 2 \mu\text{l}^{-1}$ saline) was made on both the left and right side of the nucleus (four applications at 1-min intervals). 55 min after starting the infusion, the rats were decapitated. Sham-operated rats received saline only. The 5-HIAA levels are given as % of control values and represent means $\pm$ s.e.m.; number of rats are given in parentheses. Significance of difference between groups (*t*-test): * $P < 0.001$.



assumption that the psychotomimetic actions of LSD correlate with a decrease of the 5-HT turnover^{4,23} since the lisuride-induced decrease of 5-HT turnover is not at all associated with hallucinogenic effects. Therefore, the biochemical changes induced by both lisuride and LSD may solely represent an epiphenomenon unrelated to the hallucinosis. The differences between the effects of lisuride and LSD on the rat monoaminergic system are of a quantitative rather than a qualitative nature. In particular, from our results it can be concluded that the stimulation of 5-HT autoreceptors is not a prerequisite for hallucinogenic action, as has been hypothesised for various indole derivatives, including LSD^{24,25}. It cannot be excluded, however, that LSD as well as tryptamine derivatives, which are compounds of the mixed agonist-antagonist type at DA receptor sites²⁶, could induce hallucinosis by reciprocally interacting with the DA receptor¹⁶, both in its antagonistic as well as agonistic state²⁷. The DA receptors seem to participate in some way in the hallucinogenic effects of LSD, as they are antagonised by neuroleptic drugs^{28,29} which are thought to act by DA receptor blockade³⁰.

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The proton pump is a molecular engine of motile bacteria

BACTERIA move by rotation of a spiral flagellum^{1,2}. The flagellar motor¹ is thought to be a basal body consisting of a rod with four threaded rings localised in the cell wall and connected to the flagellum^{3,4}. Larsen *et al.*⁵ have found that the source of energy for motility is an intermediate of oxidative phosphorylation rather than ATP. The idea that an ion gradient is the basis of bacterial movement originates from Mitchell⁶, and has been favoured by several authors⁶⁻⁸. Some indications of the direct coupling between the H⁺ electrochemical gradient ($\Delta\bar{\mu}_{H^+}$) and motility were obtained in our laboratory when we were studying a photosynthetic bacterium, *Rhodospirillum rubrum*^{7,9,10}. We describe here experiments demonstrating that bacterial motility can be supported by enzymatically generated or artificially imposed constituents of $\Delta\bar{\mu}_{H^+}$, that is, electric potential ($\Delta\psi$) or ΔpH .

We first studied the action of a proton-phosphorus uncoupler, 3, 4-dichlorocarbonyl cyanide phenylhydrazine (CCCP) on the motility of *R. rubrum* cells in light. CCCP abolished motility within less than 4 s, which agrees with the results of Larsen *et al.* obtained with *Escherichia coli*. Addition of oligomycin, an inhibitor of *R. rubrum* membrane ATPase, and of F⁻, an inhibitor of membrane pyrophosphatase, did not modify the effect of CCCP (Fig. 1). Thus the action of CCCP can be explained by inhibition of $\Delta\bar{\mu}_{H^+}$ rather than by exhaustion of ATP or pyrophosphate. A similar result was obtained when 1 $\times$ 10⁻⁴ M dicyclohexylcarbodiimide was used instead of oligomycin (data not shown). The concentration of cellular ATP decreased more slowly than the motility (Table 1a), also in agreement with the data for *E. coli*.

To investigate whether the concentration (ΔpH) or electrical ($\Delta\psi$) components of $\Delta\bar{\mu}_{H^+}$, or both, can support motility, cells were incubated with valinomycin, which decreases $\Delta\psi$ due to K⁺-conductivity, or with acetate, which penetrates the mem-

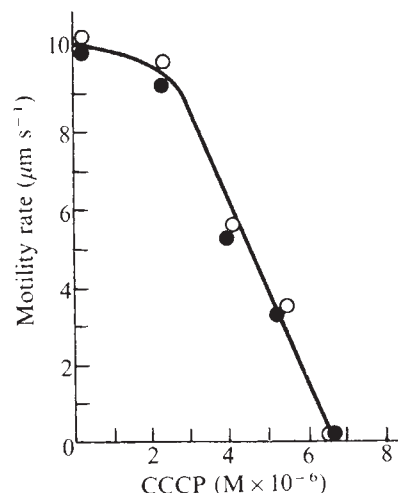


Fig. 1 The CCCP inhibition of *R. rubrum* motility. Bacteria were grown as described elsewhere¹⁰. The incubation medium contained 10 mM potassium phosphate (pH 6.8) and 0.1 mM EDTA. One ml of the suspension contained 1 $\times$ 10⁷ cells. The motility rate was estimated microscopically, according to Shoemith¹¹. The concentration of cells was determined in a counting chamber. ○, in the presence of oligomycin (10 μg ml⁻¹) and 10 mM NaF; ●, without these inhibitors.

brane in the protonated form and lowers ΔpH . Table 2 shows that the addition of valinomycin or acetate separately only slightly affects the motility of *R. rubrum* in the light, while their simultaneous addition arrests the movement of the cells. This can be understood if one takes into account the fact that the proton pumps of the bacterial membrane are in equilibrium with the total $\Delta\bar{\mu}_{H^+}$, so that a lowering of $\Delta\psi$ results in a synchronous rise of ΔpH , and vice versa. In fact, the addition of acetate increased $\Delta\psi$, which was monitored by accumulation of a permeant cation, tetraphenylphosphonium (TPP⁺), by *R. rubrum* (data not shown). As Table 2 shows, TPP⁺ has the same effect on *R. rubrum* motility as valinomycin plus K⁺. The level of cellular ATP was only slightly reduced in the presence of acetate and/or valinomycin, (Table 1b).

When light-induced uptake of penetrating cations (a probe for membrane potential¹²) and the rate of bacterial motility were studied as a function of concentration of inhibitors of the photosynthetic redox chain, *o*-phenanthroline (Fig. 2a) or antimycin (Fig. 2b), a good correlation was observed. There was no threshold value of membrane potential supporting motility (unlike ATP synthesis, which ceases at $\Delta\bar{\mu}_{H^+}$ (180 mV (ref. 14)).

Artificial $\Delta\psi$ formation was achieved by the addition of valinomycin to *R. rubrum* cells growing in the presence of K⁺ and then suspended in a K⁺-free medium. Under these conditions, K⁺ efflux results in $\Delta\psi$ ('minus' inside the bacterium). Anaerobically grown cells with K⁺ were washed in a K⁺-free medium and incubated in the presence or absence of metabolic

Table 1 Concentration of ATP (nmol per mg of dry weight) in *R. rubrum* cells under different conditions

Experiment a	Without additions 7.0	1 $\times$ 10 ⁻⁵ M CCCP, 10 s incubation 6.7	5 $\times$ 10 ⁻⁶ M CCCP, 30 min incubation 2.6
		2 $\times$ 10 ⁻² M acetate, 5 min incubation 5.4	2 $\times$ 10 ⁻² M acetate 2 $\times$ 10 ⁻⁵ M valinomycin, 5 min incubation 4.2
Experiment b	Without additions 5.8	2 $\times$ 10 ⁻² M acetate, 5 min incubation 5.4	2 $\times$ 10 ⁻⁵ M valinomycin, 5 min incubation 4.8

a, Bacteria prepared as in Fig. 1 were incubated under illumination and permanent stirring. The samples were fixed by 1 N HCl (final concentration)¹², and ATP was measured enzymatically after neutralisation, with the hexokinase and glucose-6-phosphate dehydrogenase system.

Table 2 The effect of acetate, valinomycin and tetraphenylphosphonium (TPP⁺) on *R. rubrum* motility

Incubation (min)	Additions	Motility (μs^{-1})
5	—	30.0
	$2 \times 10^{-5}\text{M}$ valinomycin	25.4
	$2 \times 10^{-2}\text{M}$ acetate	23.2
20	$2 \times 10^{-5}\text{M}$ valinomycin + + $2 \times 10^{-2}\text{M}$ acetate	0
	—	14.0
	$1 \times 10^{-4}\text{M}$ TPP ⁺	8.4
	$1 \times 10^{-4}\text{M}$ TPP ⁺ + + $2 \times 10^{-2}\text{M}$ acetate	0

Conditions as in Fig. 1.

inhibitors in light. Figure 3a (solid line) shows that the addition of valinomycin caused a strong stimulation of motility, initially very low due to the absence of available energy. The effect disappeared within two minutes. The addition of valinomycin to cells incubated in the presence of KCl and without inhibitors lowered the motility rate (Fig. 3a, broken line).

In a further experiment (Fig. 3b, solid line), the motility rate increased 4-fold when the pH was changed from 9.3 to 6.1 (a permeant anion SCN⁻ was used to abolish the diffusion potential formed by the H⁺-influx). The opposite effect was obtained by alkalisating the bacterial suspension from pH 6.1 to 9.3 (without inhibitors), leading to a transient depression of motility. The above data thus indicate that the motility of *R. rubrum* can be supported by $\Delta\bar{\mu}_{\text{H}^+}$ with no ATP involved. The motility is not mediated by $\Delta\psi$ or ΔpH -driven transfer of K⁺ or Na⁺, because the light-supported motility is observed for a long time in distilled water.

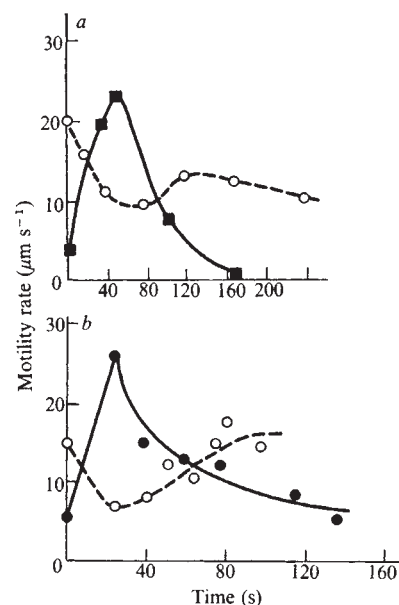


Fig. 3 Motility driven by an artificially imposed electric potential (a) or pH gradient (b). a, Cells were washed twice with 10 mM MOPS-Tris, 0.1 mM EDTA (pH 6.8) and incubated in this medium, supplemented alternately with oligomycin ($10 \mu\text{g ml}^{-1}$) and 10 mM NaF (solid line), or with 50 mM KCl (broken line) $9 \mu\text{g ml}^{-1}$ of a bacterial suspension and $1 \mu\text{l}$ of $1.67 \times 10^{-4}\text{M}$ valinomycin solution in 50% ethanol were separately placed on the same slide of the cell-counting chamber. The drops were mixed at zero time and the rate of motility was measured in 10–15 s. b, Broken curve, bacteria were preincubated for 5 min in the presence of 10 mM MOPS-Tris, 0.1 mM EDTA, 1 mM KSCN, pH 6.1. At zero time, $9 \mu\text{l}$ of the same medium, pH 9.3, was added to $1 \mu\text{l}$ of bacteria, solid curve; bacteria were preincubated in the above medium, pH 6.8, for 25 min in the presence of $1 \times 10^{-5}\text{M}$ antimycin, oligomycin ($10 \mu\text{g ml}^{-1}$), 10 mM NaF, and then the pH was adjusted to 9.3 and another 5 min incubation followed. $9 \mu\text{l}$ of the same medium, pH 6.1, were added to $1 \mu\text{l}$ of bacteria at zero time.

The mechanism by which H⁺ moves down the H⁺ electrochemical gradient activating the flagellar motor is obscure. Probably, it is the M-ring of the basal body localised in the cytoplasmic membrane⁴ that is primarily involved in the energy-transducing process. Figure 4 shows a scheme illustrating a possible mechanism of the $\Delta\bar{\mu}_{\text{H}^+}$ -supported M-ring rotation. Let us suppose that there is a ribbon of proton-acceptor groups (say, $-\text{NH}_2$ -groups) on the M-ring. One of the amino groups opens into the upper proton-conducting path. An anionic group, for example, a carboxy group, is located at the beginning of lower proton-conducting path. The protonation of the amino group leads to electrostatic attraction between the $-\text{NH}_3^+$ and $-\text{COO}^-$ -groups. Since the $-\text{NH}_3^+$ is attached to the M-ring, the latter must turn to bring the $-\text{NH}_3^+$ and $-\text{COO}^-$ -groups together. The distance between the two proton-conducting pathways is postulated to be equal to that between two neigh-

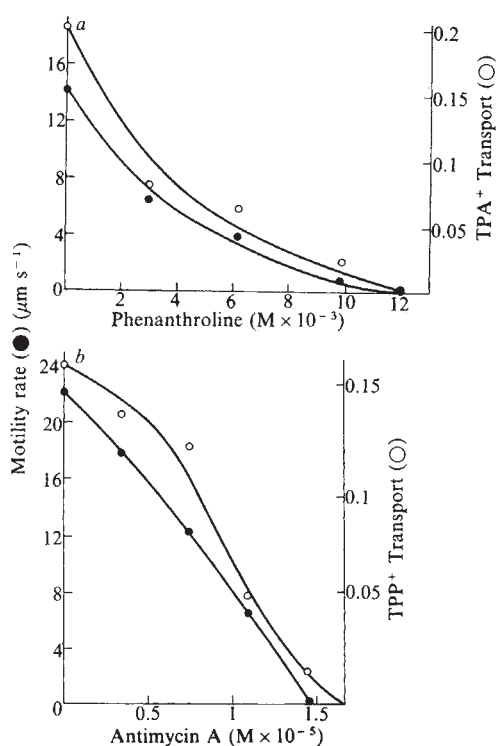
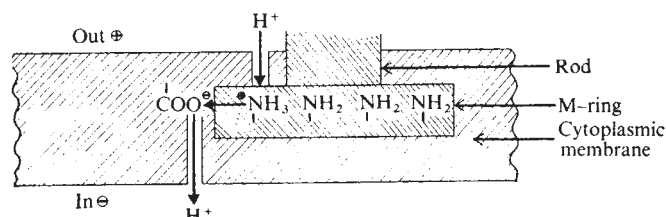


Fig. 4 A model for the rotation of the basal body. See text for explanation.



bouring amino groups. Thus, the movement of the M-ring results in the next $-NH_2$ -group being transferred to the upper path. The protection of $-COO^-$ by $-NH_3^+$ involves an extrusion of H^+ into the cytoplasm by the lower path.

It should be noted that M. D. Manson *et al.* have published results of their experiments with a diplococcus; these are in very good agreement with the above data for *R. rubrum*.

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GTPase activity at ends of microtubules

MICROTUBULES are involved in many important biological processes, including cell motility, cell division, morphogenesis and axonal transport, and it is of fundamental interest to understand the mechanism and regulation of tubulin polymerisation in order to clarify their function. Previously we presented evidence that brain tubulin, devoid of microtubule associated proteins (MAPs) after fractionation by phosphocellulose chromatography, exhibits a characteristic GTPase activity which is polymerisation-dependent and can be attributed to tubulin itself¹. A detailed analysis of the steady-state GTPase activity led us to propose a model, according to which the rate of the polymerisation-dependent GTP hydrolysis should be proportional to the product of the concentration of microtubule ends and of tubulin-GTP. At the polymerisation equilibrium, the concentration of tubulin dimers is just equal to the 'critical concentration'², which is the minimum concentration above which tubulin polymers exist. Therefore, at the polymerisation equilibrium, the rate of GTP hydrolysis should be directly proportional to the concentration of microtubule ends. We now present evidence consistent with this prediction, from experiments in which microtubules were fragmented by sonication to increase the number of microtubule ends and the resulting change in the rate of GTP hydrolysis was measured.

We could not use microtubules purified by polymerisation-depolymerisation cycles³, as these microtubules have been shown to carry with them several MAPs^{4,5}, which (as stated above) we have shown to exhibit a GTPase activity of their own¹, independent of the polymerisation reaction. Nor could we use microtubules polymerised in the presence of a polycation such as DEAE-dextran⁶ or in high Mg^{2+} concentrations and glycerol⁷, in place of MAPs, since these

Table 1 Effect of sonication on microtubule length distribution and on the steady state GTPase activity of microtubule suspensions

t_s (s)	V_{ts} ($\mu\text{mol min}^{-1}$)	V_{ts}/V_0	i (μm)	i_0/i_{ts}
0	0.022	—	3.9	—
3	0.048	2.2	1.5	2.6
9	0.079	3.6	0.9	4.3

t_s , sonication time (s). Microtubule suspensions were subjected to sonication 30 min after induction of the polymerisation reaction by a temperature jump from 4 °C to 37 °C. V_{ts} , initial rate of GTP hydrolysis after sonication. V_0 , rate of GTP hydrolysis of non-sonicated sample. i , average length of microtubule in μm . i_0/i_{ts} , ratio of the average length of microtubules before sonication to that after sonication.

microtubules appeared to be mostly in bundles and were very difficult to identify individually and to count. The best system we found to perform the sonication experiments consisted of microtubules reconstituted from phosphocellulose-purified tubulin and boiled MAPs. MAPs, boiled for 5 min in a medium containing 0.75 M NaCl and 2 mM dithiothreitol, have been shown to be active in promoting microtubule assembly⁸, and we have observed that they no longer exhibit GTPase activity. In addition, microtubules reconstituted with boiled MAPs appeared as singles under the electron microscope and could be easily measured and counted.

The preparation of tubulin and boiled MAPs was subjected to sonic vibration after the polymerisation equilibrium had been established (after 30 min at 37 °C). After sonication, it was confirmed by centrifugation and turbidimetry that no polymerisation had occurred, and that the turbidity of the sonicated samples was fully reversible when the temperature was lowered to 4 °C. As the experiments were performed at polymerisation equilibrium and as no apparent depolymerisation occurred during the sonication time, the weight concentration of microtubules was identical before and after sonication. This means that one can compare directly the average length of microtubules before and after sonication, and this should be inversely proportional to the total number of microtubules (or of microtubule ends).

Figure 1a, b and c are electron micrographs and microtubule length distribution diagrams of microtubule preparations before and after two different times of sonication. Figure 2 shows the change in GTPase activity of microtubule preparations after sonication. The results indicate that the shortening of microtubules causes an increase in GTPase activity.

A good correlation is obtained between increasing GTPase activity and decreasing average length of microtubules, that is, increasing number of microtubule ends (Table 1). In addition, the rate of GTP hydrolysis apparently does not

Table 2 Evolution of GTPase activity and length distribution after sonication

Δt (min)	$V\Delta t_i$ ($\mu\text{mol min}^{-1}$)	$V_0/V\Delta t_i$	i (μm)	$i_{\Delta t}/i_0$
0	0.079	—	0.9	—
30	0.049	1.6	1.4	1.55
50	0.037	2.1	1.5	1.7

Δt , time after 9 s sonication time (temperature 37 °C). $V\Delta t_i$, rate of GTP hydrolysis at different times after sonication, $V_0/V\Delta t_i$, ratio of the rate of GTP hydrolysis directly after sonication to that at different times after sonication.

remain constant after sonication (Fig. 2), but decreases slightly with time. Electron microscopic observation shows that the average length of the microtubules increases (Fig. 1d, Table 2), confirming that the GTPase activity of microtubule solutions varies with the number of microtubules.

Thus, the results of the sonication experiment reported here give strong support to the model that we have proposed concerning the relationship between GTPase activity of tubulin and the polymerisation reaction¹—that GTP hydrolysis occurs as a tubulin subunit polymerises onto the end of a microtubule. Generally, the rate of GTP hydro-

lysis will be determined by the frequency of tubulin-tubulin association of the type which occurs during microtubule growth, during the nucleation process or at the polymerisation equilibrium. Therefore, measuring the inorganic phosphate release linked to the self-assembly process should provide a means of measuring the number of tubulin-tubulin interactions.

At the polymerisation equilibrium, where the rate of microtubule-tubulin associations balance exactly with the rate of dissociation of tubulin subunits from the end of the microtubules, the rate of GTP hydrolysis directly reflects

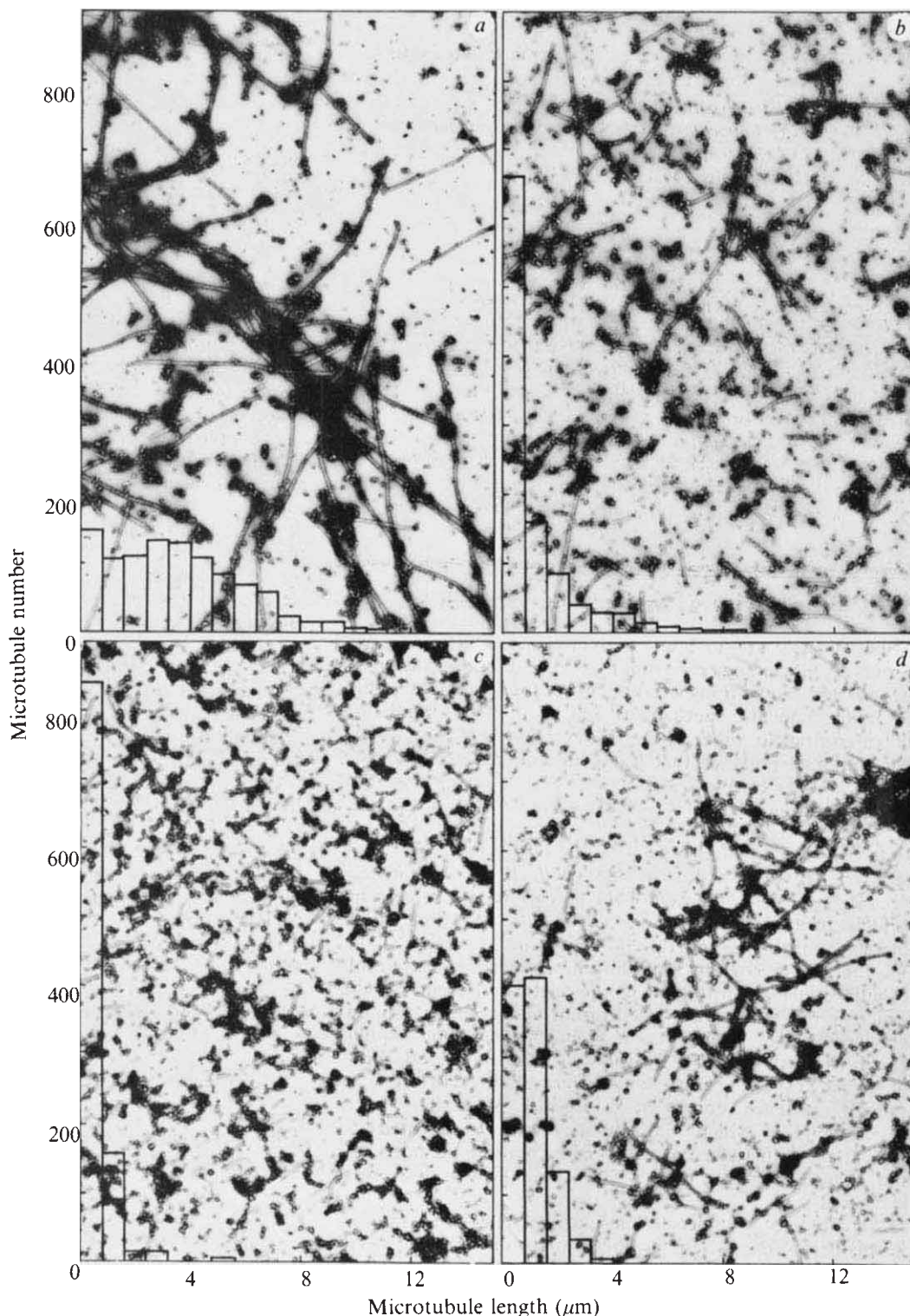


Fig. 1 Electron micrograph and length distribution of microtubule preparations. Phosphocellulose purified tubulin (1.3 mg ml^{-1}) was induced to polymerise in the presence of boiled MAPs (0.6 mg ml^{-1}) at 37°C in reassembly buffer containing 0.05 M Mes ($2\text{-}N$ -morpholino) ethane sulphonic acid) at $\text{pH } 6.6$ adjusted with NaOH , 0.5 mM EGTA, 1 mM MgCl_2 and 0.5 mM GTP. After 30 min at 37°C , two samples were sonicated respectively for 3 s and 9 s at setting 10 with a Bio-sonick III sonicator. Electron micrographs and the length distributions are given for samples before sonication (a), immediately after 3 s sonication (b), immediately after the 9 s sonication (c) and 50 min after the 9 s sonication (d). Between 200 and 500 microtubules were measured and counted for each sample, and the resulting distributions were normalised to a total of $1,000$ microtubules.

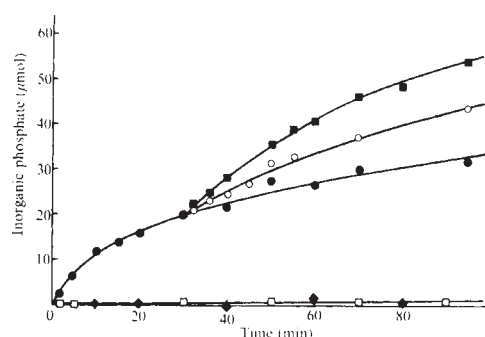


Fig. 2 Kinetics of GTP hydrolysis under the conditions of Fig. 1. (●) Unsonicated sample; (○) 3 s; (■) 9 s sonicated samples. (◆) and (□), respectively, show the non-significant GTPase activity exhibited by the boiled MAPs and the phosphocellulose purified tubulin.

the frequency of exchange of tubulin subunits at the ends of the microtubules. Knowledge of such a relationship between GTPase activity of tubulin and the polymerisation reaction should permit us to investigate the mechanism and the regulation of tubulin polymerisation *in vitro* and *in vivo*.

For example, the rapidity of subunit exchange at the polymerisation equilibrium should be related to the dynamic stability of the microtubules, and the steady-state rate of GTP hydrolysis should also reflect this dynamic stability. It has been reported⁹ that MAPs stabilise microtubules by slowing down the rate of microtubule depolymerisation. Also, the critical concentration for polymerisation in the presence of Mg^{2+} (ref. 7) is somewhat higher than that in the presence of MAPs². We found that the GTPase activity of solutions of microtubules which polymerised in the presence of Mg^{2+} and glycerol¹ was three times higher than that of solutions of microtubules polymerised in the presence of MAPs. According to our model, and with equal length distribution, this would also indicate a greater stability of MAP-polymerised microtubules as compared to microtubules polymerised in the presence of Mg^{2+} and glycerol. Finally, according to the model proposed, the suggestion that the rate of GTP hydrolysis should be directly proportional to the concentration of microtubule ends is valid only at the polymerisation equilibrium. The observed redistribution of microtubule length after sonication, however, shows that, in these conditions, the number and size of polymers are not yet at equilibrium even if the concentration of dispersed monomers retains approximately its equilibrium value. This is a situation of approaching equilibrium, that is, the rate of polymerisation is nearly equal to the rate of depolymerisation. This situation has been analysed by Oosawa¹⁰; in our case, however, the difference in rate must be very small and so does not alter our conclusion.

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5-Bromodeoxyuridine induces expression of a tumour-specific surface antigen on normal avian cells

TUMOUR specific surface antigen (TSSA) has been described on all cell types transformed *in vivo* or *in vitro* by avian sarcoma virus (ASV)^{1,2}. It is distinct from the viral structural antigens and seems to be expressed only on morphologically transformed cells. However, it can also be present exceptionally on untransformed cells infected at non-permissive temperature with ASV ts mutants³. We present here evidence that treatment of normal chick embryo fibroblasts (CEF) and Japanese quail embryo fibroblasts (JQEF) with 5-bromodeoxyuridine (BRdU) induces a new surface antigen which reacts with rabbit serum to ASV-transformed rat XC cells. This antigen is related to TSSA induced on both ASV-transformed mammalian cells and CEF.

The induction of a cell-surface antigen related to TSSA was studied on embryonic fibroblasts of various avian species treated for 15 d with BRdU ($4 \mu\text{g ml}^{-1}$) and they were compared with CEF and mammalian fibroblasts transformed by Rous sarcoma virus (RSV). TSSA expression was measured by a mixed haemadsorption test⁴ which is specific for ASV-induced TSSA^{5,6}. As Table 1 shows, this test was positive with both CEF productively infected with different RSV strains and with non-permissive rat and hamster cells transformed by the same strains, whether or not viral antigens were expressed at their surface. Moreover, the test was negative with CEF productively infected with the helper virus RAV1 and with hamster cells transformed by adenovirus 12 or by chemical carcinogens (data not shown).

Cells were seeded sparsely on coverslips and incubated with TSSA-specific anti-XC cell rabbit serum, and then with sheep red blood cells (SRBC) coated with rabbit anti-SRBC serum and goat anti-7S rabbit globulin. Both CEF and JQEF, after 2 weeks in the presence of BRdU, expressed a surface antigen cross-reacting with anti-XC serum, whereas duck embryo fibroblasts (DEF) remained negative (Table 2), and BRdU-treated cells expressed less antigen than ASV-transformed CEF and mammalian cells.

The difference in quantitative expression of the antigen could have resulted if the TSSA expressed on ASV-transformed cells were not a single antigen, but a complex consisting of at least two antigens. In that case, the antigen expressed on BRdU-treated cells could have consisted of only one of the determinants. To check this possibility, extensive cross-absorption experiments were performed. The results (Table 3) suggest that there are at least two antigenic determinants of ASV-induced TSSA, one common to ASV-transformed CEF and mammalian cells and BRdU-treated CEF, and the other specific to ASV-transformed cells.

A possible re-expression of embryonic antigens able to cross-react with anti-XC serum in both BRdU-treated CEF and ASV-transformed cells was investigated by extensive absorption of rabbit anti-XC serum with a homogenate of 10-d-old chick embryos followed by testing of the absorbed serum against BRdU-treated CEF and ASV-transformed

Table 1 Comparative expression of TSSA and viral proteins on different transformed cell lines

Cells	Virus	Subgroup	Cell phenotype ¹	TSSA	Membrane expression of gp85	p prot.
CEF	SR-RSV	A, D	T	+	+	+
CEF	B-RSV (RAV1)	A	T	+	+	+
CEF	PR-RSV	C	T	+	+	+
CEF	RAV1	A	NT	—	+	+
Rat XC	PR-RSV	C	T	+	+	+
Rat TWERC	PR-RSV	C	T	+	—	—
Hamster RS2	SR-RSV	D	T	+	+	+
RB 12/5	B-RSV (RAV1)	A	T	+	—	—

T, transformed; NT, not transformed.

*Including p27.

†Other than p27 (compare ref. 7).

cells (Table 3). No diminution in the positive reaction was observed with any cell type. However, this experiment did not rule out the possibility that the antigen induced by BRdU and ASV-induced transformation is a very early embryonic antigen. Experiments are in progress to test this possibility.

So far, TSSA has been considered a specific marker for ASV-induced cell transformation, because all ASV-transformed cells, either avian or mammalian, express TSSA. Moreover, quail cells transformed by murine sarcoma virus do not express TSSA⁸. But here we report that incubation of normal CEF with BRdU induces the expression of at least one determinant of TSSA in the absence of ASV. As already noted^{9,10}, BRdU elicits changes in cell morphology consisting of an enlargement and flattening of the cytoplasm, which are clearly different from transformation by ASV in which the cells become rounder, detached from neighbouring cells and able to grow in three dimensions. However, BRdU elicits an increase of cellular agglutinability by concanavalin A comparable with that observed after ASV-induced transformation¹¹. Thus the appearance on BRdU-treated cells of an antigen related to a deter-

minant of TSSA together with enhanced lectin agglutinability may be considered a BRdU-induced step towards transformation.

We investigated whether the expression of TSSA in BRdU-treated cells could be related to some modification of the expression of the endogenous virus of CEF, notably expression of the viral p proteins which can appear at the cell surface¹². Indeed, endogenous type C viruses have been induced efficiently by BRdU in growing mouse embryo fibroblasts¹³, and in CEF the production of inducible virus seemed to be a unique characteristic of some but not all pedigrees of CEF¹⁴. In the CEF used in our investigation no virus was induced after BRdU treatment. However, immunofluorescence tests on fixed cells with a monospecific

Table 2 Expression of TSSA on BRdU-treated avian and ASV-infected chicken or mammalian cells

Cells	Normal rabbit serum	Rabbit anti-XC ⁺ serum		
		1/20	1/40	1/80
CE-BL	—	—	—	—
CE-BL + BRdU	—	+++	+++	+
CE-C	—	—	—	—
CE-C + BRdU	—	+++	+++	+
CE-BL + ASV	—	+++	+++	+
JQEF	—	—	—	—
JQEF + BRdU	—	+++	++	—
DEF	—	—	—	—
DEF + BRdU	—	—	—	—
BHK21/13	—	—	—	—
TWERC	—	+++	+++	+++
RBH	—	+++	+++	+++

TSSA were detected by a mixed haemadsorption test. The degree of haemadsorption was estimated as follows: + + +, test cells completely covered with indicator red blood cells; + +, cells moderately covered; +, cells covered with scattered indicator red blood cells. XC, cell line derived from a rat sarcoma produced by ASV; CE-BL, chick embryo fibroblasts from gs⁺ embryos; CE-C, chick embryo fibroblasts from gs⁻ embryos; JQEF, Japanese quail embryo cells; DEF, duck embryo cells; BHK21/13, normal hamster cell line used as a control; TWERC, non virogenic rat cell transformed by ASV; RBH, virogenic hamster cell deriving from a hamster tumour by ASV.

Table 3 Adsorption of rabbit anti-XC serum with BRdU-treated and ASV-transformed CEF

Cells	Rabbit anti-XC	Antisera	
		Rabbit anti-XC absorbed on CE-BL + BRdU	Rabbit anti-XC absorbed on CE-BL + ASV
CE-BL	—	—	—
CE-BL + BRdU	++	—	—
CE-BL + ASV	++	+	—
TWERC	+++	++	++
RBH	+++	++	++

Residual TSSA activity was measured using the technique described in Table 2, after absorption on BRdU-treated or ASV-transformed CEF. For details of cells see Table 2.

hyperimmune serum against the viral p27 protein revealed increased expression of this protein in the BRdU-treated CEF (Table 4), whereas no expression of p27 was observed in BRdU-treated JQEF and DEF which do not carry the type-specific antigen of subgroup E of ALV (chf). Enhancement of the expression of gs antigen(s) has also been noted in virogenic ASV-transformed mammalian cells¹⁵.

Our results show that treatment of normal CEF and JQEF with BRdU elicits the appearance of an antigen related to ASV-specific TSSA expressed on ASV-transformed chicken and mammalian cells. They further suggest that ASV-specific TSSA is a complex of at least two antigens, one of which is related to the BRdU-induced antigen. The nature of the second antigen and its significance remain to be determined. Two models at least can be proposed. First, because normal CEF and JQEF carry a gene closely related, in molecule hybridisation experiments,

Table 4 Expression of viral p27 protein on BRdU-treated and normal CEF, JQEF and DEF

Cells	Degree of fluorescence		Degree of fluorescence		Degree of fluorescence	
	100%+++	50%+++	100%++	50%++	100%+	50%+
CE-BL	20	20	40	80	80	160
CE-BL + BRdU	40	160	80	640	1280	2560
CE-C	20	20	20	20	40	80
CE-C + BRdU	20	40	640	1280	2560	2560
JQEF	20	20	20	20	20	20
JQEF + BRdU	20	20	20	20	20	20
DEF	20	20	20	20	20	20
DEF + BRdU	20	20	20	20	20	20

The figures are the reciprocal of the serum dilution giving the indicated degree of fluorescence in 100% or 50% of the cells. Cells were seeded sparsely on coverslips in Leighton tubes which were kept at 37 °C. One day later they were washed with phosphate-buffered saline, fixed with acetone for 10 min, incubated for 45 min at 37 °C with increasing dilutions of the monospecific rabbit anti-p27 ASV immune serum and incubated for 30 min at room temperature with fluorescein conjugated globulin anti-rabbit 7S IgG. + + +, Intense continuous fluorescence; ++, intense granular fluorescence; +, moderate granular fluorescence. For details of cells see Table 2.

to the viral *src* gene responsible for cell transformation¹⁶, the BRdU-induced TSSA could be the protein coded by the cellular *src* (sarco) gene. In this case, BRdU could induce the expression of the protein, which is normally repressed in untreated cells. The lack of reactivity of the anti-XC antiserum with BRdU-treated DEF could be due to the antigenic unrelatedness of the products of endogenous *src* of DEF and CEF. Experiments using specific antisera to BRdU-treated and untreated DEF are in progress to test this hypothesis. Second, the TSSA-related antigen(s) expressed on BRdU-treated CEF and JQEF could be unrelated to the antigenic determinant coded by the viral *src* gene and related to another cellular determinant of ASV-specific TSSA. This determinant could be a cross-reacting antigen common to avian and mammalian cells and expressed after both BRdU treatment and virus-induced transformation—for instance, an early embryonal antigen. Because BRdU treatment also enhanced the expression of p proteins in treated CEF, the mechanism of induction of the TSSA-related antigen might also be the same as that of induction of the cryptic chick helper factor.

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Errata

In the letter by R. S. Bradley and J. England, *Nature* **271**, p. 736, the title should read: Influence of volcanic dust on glacier mass balance at high latitudes. In paragraph 4, line 4, (TxMDD) should read (TxMDD). In paragraph 5 the equations in lines 7 and 8 should read: $y = -2.79x + 224.8$ and $r = -0.94$. In line 26 for 1933–64 read 1963–64. In paragraph 7, for $P < 0.01$ read $P < 0.001$. Figure 4 legend should include an extra line: Asterisks indicate data for ≤ 1 month estimated.

In the letter 'Gating properties of acetylcholine receptor in newly formed neuromuscular synapses' by H. R. Brenner & B. Sakmann *Nature* **271**, p. 366, the time calibration bar in Fig. 2 should be marked 20 ms, and that in Fig. 3 should read 2 ms.

In the letter 'Regeneration of endoderm by ectoderm isolated from mouse blastocytes' by R. Pederson, *Nature* **270**, 435, in paragraph 2 line 3 for 'thin inner cell masses' read 'then inner cell masses'.

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matters arising

Corrected age of the Pliocene/Pleistocene boundary

THE recent report of Haq *et al.*¹ prompts the following criticisms.

Gephyrocapsa oceanica first appeared shortly after the Olduvai event in cores RC11-220, CH61-171, V16-205 and V12-18 (their Fig. 1). In the Le Castella type section it appeared well below the 'marker bed' (their Fig. 2), but in the compilation (their Fig. 5) it starts at the base of the 'marker bed'. Also *Globigerinoides obliquus* became extinct just after the 'marker bed' in the Le Castella section but in the compilation (their Fig. 5) it became extinct at or just below the 'marker bed'.

The following problems remain in spite of the slightly misleading title of their paper: first, there seems to be no reliable palaeomagnetic or radiometric date on the type Calabrian and until these data are forthcoming, unreliability will surround the positioning of the Pliocene–Pleistocene boundary.

Second, Haq *et al.* have failed to make a convincingly accurate correlation of the biostratigraphic events in the type section at Le Castella with some selected deep-sea cores. This is not surprising because in the Le Castella section two key species have stratigraphic ranges which clearly overlap *G. obliquus* and *G. oceanica*—while in all their examined deep-sea cores there is no such overlap. Is the Le Castella section therefore biostratigraphically atypical? Are Haq *et al.* hammering a square golden peg into a round hole at the top of the Olduvai event to make things fit? If the 'marker bed' at the Le Castella section is to be fixed by a golden peg then it will present us with nearly insurmountable problems of correlation. Therefore serious consideration must be given to the abandonment of the type section.

What we need is the judicious selection of a land section of marine rocks covering a period of 1–3 Myr which contains reasonably good microfossil fauna and flora as well as sediments which can be palaeomagnetically dated. With regard to this, we await the results of the long deliberations of the INQUA subcommission on the Pliocene/Pleistocene boundary with mounting impatience.

Meanwhile, Selli's² estimate of the Pliocene/Pleistocene boundary of 1.85 Myr is apparently just as valid as the

Haq *et al.* estimate of 1.6 Myr; but neither is scientifically acceptable.

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HAQ *et al.* REPLY—Jenkins draws attention to the inconsistency between some of the deep-sea cores and the Calabrian sections that we studied¹, in that the upper stratigraphic range of *Globigerinoides obliquus* is not the same relative to the lower stratigraphic range of *Gephyrocapsa oceanica*. He concludes that we have "... failed to make a convincingly accurate correlation of the type section at Le Castella with some selected deep-sea cores ..."

Sedimentation rates at Le Castella were moderately high, so that the 25–30 m of upward displacement of the *G. obliquus* LAD (last appearance datum) at Le Castella relative to its position in the biostratigraphy in the deep-sea cores is of the order of 0.1 Myr in equivalent time. The stratigraphic range limits of 10 other taxa that appear in more than one of the cores we studied (see our Fig. 1) show variations of comparable or greater (chronological) magnitude in several instances, but most experienced micropalaeontologists would hardly be surprised at this. Local discrepancies of this degree are the 'noise' or uncertainty that is inherent in biostratigraphy. Nor should it be necessary to point out that the observed last occurrence of any fossil, microfossils in particular, is more liable to vary from one place to another than its first appearance, due either to isolation of relict populations or to upward reworking.

It is, therefore, far more significant that the FAD (first appearance datum) of *G. oceanica* invariably occurs immediately above that of *G. caribbeanica* in the deep-sea cores and also at Le Castella, and that in every one of the cores in which these closely spaced FADs were observed, they bracket the upper boundary of the Olduvai Event. This is unequivocal evidence for the close correlation of the top of the Olduvai Event to the Pliocene/Pleistocene boundary, because the 'marker bed' at Le Castella is only 25–30 m higher than this pair of FADs in the boundary–stratotype section.

In view of the internal consistency of the combined planktonic microfossil data with a calibration of the boundary to an age of 1.6 Myr, we would answer Jenkins' rhetorical question: no, the slight overlap of the range of *G. obliquus* and *G. oceanica* at Le Castella but not in two deep-sea cores does not appear to be 'biostratigraphically atypical' any more than a four-leaf clover is biologically atypical.

Jenkins also wonders why we show the *G. oceanica* FAD and the *G. obliquus* LAD at Le Castella differently in two of our Figs. Our Fig. 2 represents the biostratigraphy as we found it in our samples from Le Castella. In Fig. 5, however, we depicted the 'marker bed' as a disproportionately thick band, equivalent to 0.05 Myr, to suggest our limits of biostratigraphic certainty and thus to give our summary a higher degree of probability. Therefore, the two datum events are pictured to coincide at the base of this 'fuzzy zone' because we cannot be certain that the true range of *G. obliquus* extends any higher, despite our observation. We regret that this change in viewpoint was not explained in detail.

We are accused of unscientifically trying to make the palaeontological data fit our preconceptions, which Jenkins describes as an attempt to force a round golden peg into a square hole. On the contrary, our philosophy is that gold is where you find it: our only preconceptions were that the boundary is already defined by the 'marker bed' at Le Castella, and that more micropalaeontological data from the critical Calabrian sections would help to resolve obvious anomalies in the published accounts that focused on fewer taxa. Dr Jenkins' preconception is apparently that all this effort is wasted because the Le Castella and Santa Maria di Catanzaro sections do not have palaeomagnetic control. We note in this context unceasing problems that have plagued palaeomagnetic correlations of the antipodeal Neogene in the absence of adequate micropalaeontological control^{2,3}. The use of multiple overlapping biochronological criteria, which demonstrably reduces or overcomes the biostratigraphical 'noise' in correlation, is the fundamental tool in linking palaeontological and geophysical data in a stratigraphic time-scale⁴. To seize upon inconsistency in the range of one element in such a synthesis as evidence that we do not know what we

are doing, or to compare the validity of our results with that of Selli's estimate⁵ based on poorly-founded assumptions of subsidence rates in the Po Valley, is incompatible with standard geochronological procedures.

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The number of ancestral genes contributing to a sample of B8 alleles

AN interesting problem raised by Arnason *et al.*¹ is that of the number of distinct genes, at any given point in history, ancestral to a given currently observed set. If this can be estimated, the total number of meioses at which an observable recombination between a B8 allele at the HLA-B locus and S allele at the Bf locus could have occurred (but has not) may be obtained, and an upper bound may then be placed on the recombination fraction between the two loci. Arnason *et al.*¹ obtained an 'estimate' of 2,000 meioses, based on 'minimum plausible estimates' of ancestral gene numbers, but these latter were presented only as being 'not unreasonable' and 'highly conservative'. The following approach provides a method of estimation based on the evolutionary and genetic structures of the populations involved.

By analogy with concepts of effective number of alleles², and effective numbers of parents³, for any set of n homologous genes of the same allelic type we may define the effective number of ancestral genes t generations ago as the inverse of the probability that two such genes chosen randomly with replacement from the set are descended from a single gene at that time. Clearly an upper bound on the actual number of ancestors is n , attained when all the currently sampled genes are descended from distinct copies t generations ago, while the lower bound is 1. If we take into account that the same gene could be chosen twice, the required number becomes $(1/n) \div (1 - 1/n)\pi_t$ where π_t is the probability that two distinct current genes are descended from the same ancestral gene t generations ago. This increases from 1 when $\pi_t = 1$

to n when $\pi_t = 0$, and provides a reasonable estimate of the actual number of ancestors.

Now genes of the same allelic type have higher probability of being closely related than genes chosen randomly from the population, and those selected for analysis by Arnason *et al.* were chosen precisely because these were the B8 alleles. If the probability that two homologous genes randomly chosen from the current population are identical by descent relative to t generations ago is denoted by f_t , and q is the current frequency of the B8 allele we have

$$\pi_t = \text{Prob (2 genes have the same ancestors } t \text{ generations ago | both B8)} \\ = \text{Prob (both B8 | same ancestor)} \times \\ \text{Prob (same ancestor) / Prob (both B8)}$$

$= qf_t / (qf_t + q^2(1 - f_t)) = f_t / (f_t + q - qf_t)$ Finally we may write $f_t \approx (1 - \alpha^t)$ where α is the dominant eigenvalue of the process of increasing homozygosity in a population. In any given generation $\alpha \approx 1 - 1/2N$ where N is the effective population size, and thus bounds on the value of α are given by bounds on the effective population size; this effective size of course changes over history, but over any given period an overall net value may be used.

Combining the above formulae we may estimate the number of ancestors t generations ago by

$$\text{Anc}(t) = (1 - \alpha^t(1 - q)) / (1 - \alpha^t(1 - q/n)) \quad (1)$$

The number of opportunities for recombination since T generations ago in the ancestral lines of currently sampled genes is then

$$\text{Seg}(T) = \sum_{t=0}^{T-1} \text{Anc}(t)$$

and approximating this sum by the corresponding integral we have

$$\text{Seg}(T) = T + q(1 - 1/n) \log_e(\alpha^T + (n/q) \times (1 - \alpha^T) / (1 - q/n)) - \log_e \alpha \quad (2)$$

which has the correct upper and lower bounds of T when $\alpha = 0$ and nT when $\alpha = 1$. Thus we may estimate, albeit by way of several approximations, the number of ancestral B8 genes at any point in time, and the number of meioses on any branch of the evolutionary tree of Arnason *et al.*, if estimates of effective population size and B8 allele frequency are available.

Figure 1 shows the tree form used by Arnason *et al.* Although this tree form was designed to err on the 'conservative' side, rather than to be an accurate estimate of historical events, we shall use the same tree form, but we use the method described above to estimate the total number of meioses on the tree. Estimates, based on equation (1), of the total number of ancestors at each node,

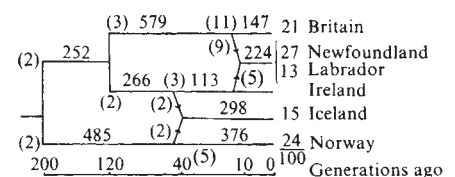


Fig. 1 Estimated numbers of meioses on each branch of the evolutionary tree, and estimated numbers of ancestral genes (in parentheses) at each node.

and on equation (2) of the number of meioses are given in Fig. 1. Although successive computations are based on exact figures, all the estimates are rounded off to the nearest integer in the diagram, these integers being, of course, only an approximate estimate of the true values. The current pooled B8 allele frequency over the relevant populations is 0.1 (J. H. Edwards, personal communication), and this value is used for q , while net effective population sizes of 10^3 are assumed for all ancestral populations, this being a reasonable lower bound. The estimated total number of meioses on the tree is then the sum of the values given in Fig. 1, which is 2,740, or approximately 37% above the value of Arnason *et al.*, giving a correspondingly lower limit on the estimate of the recombination fraction.

An estimate may also be computed using, as accurately as possible, the actual historical total single-generation sizes of each ancestral population at the period corresponding to each separate segment of the tree. This provides a value of over 15,000 meioses, but an estimate of 34 ancestors of the 100 current genes 200 generations ago. This is possible, but seems implausible, and it is likely that because of historical population structure, and because current sampling has not been geographically random over the whole of the relevant populations, the effective sizes of the populations from which the current individuals may be regarded as randomly sampled are very much smaller than actual total population sizes. Indeed they may be little more than the assumed lower bound of 10^3 . Nevertheless the value of 15,000 is of interest as an upper bound, while the 'highly conservative' estimate of 2,000 of Arnason *et al.* is indeed conservative, but taking into account historical population structure, current non-random sampling, and the fact that only B8 alleles are considered, could be not much below the actual value.

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1. Arnason, A. *et al.* *Nature* 268, 527-528 (1977).
2. Crow, J. F. & Kimura, M. *Introduction to Population Genetics Theory* (Harper and Row, London, 1970).
3. Cockerham, C. *Genetics* 56, 89-104 (1967).

reviews

Models of selection

E. R. Creed

Organismic Evolution. By Verne Grant. Pp. 418 (Freeman: San Francisco and Reading, 1977.) £11.70.

Organismic Evolution was designed and written, we are told, as an advanced text. It proceeds in fairly conventional fashion from simple concepts of population genetics through aspects of natural selection and speciation to a consideration of macroevolution. The main part of the text concludes with a section on humans, including social and cultural evolution. The book is broken down into a total of 39 chapters (one of barely two pages), most of which are further subdivided into a number of sections often followed by a list of "collateral readings", wherein are duplicated, in full, items from the main bibliography. Thus, to choose but one example, the bibliographical details of the author's 1963 book, *The Origin of Adaptation*, are repeated ten times.

What seems to be lacking most of all is a synthetic approach—the development and application of the basic principles presented in earlier chapters, to the broader fields of evolution which follow. This impression is perhaps unduly strengthened by the fragmented text, with its many short subsections, the relevance of some of which, to the book as a whole, is often in doubt. For example, three pages, including a photograph, map and table are devoted to the population details of the Giant Sequoia, and yet the next subsection is on "Polymorphism"; the sequoias and whatever significance they may have are forgotten.

Although most of the book is devoted to diploid organisms, the basic model of selection is developed in terms of haploids. Whereas this may make for simplicity in some directions, it is very misleading in others, particularly in ignoring the existence of heterozygotes; this also possibly explains an earlier assertion that in balanced polymorphism, the polymorphic types are preserved by selection in favour of diversity, thus denying the idea of heterozygote advantage which is, however, dealt with elsewhere. Equally misleading is the equation of selective advantage with reproductive rate, and also the strangely restrictive interpretation placed on the term fitness. In a discussion on evolution, fitness should have a wealth of meaning

and refers to far more than just the number of progeny left by a genotype; it is *not* a "purely quantitative and operational concept". One also gets the impression that adaptive landscapes have been misinterpreted—the steeper the slope, the quicker would be the ascent.

A quotation from p112 exemplifies some of the unsubstantiated generalisations that occur from time to time: "Natural selection operates most effectively under conditions of competition; conversely, the selective pressures are relaxed when competition is absent from the scene . . .". One has only to look back a few pages to find descriptions of several situations in which selection is not dependent on competition.

The statement (p115) that "Density-dependent factors tend to have a stabilizing effect on population number" is perhaps a truism when the factor affects the growth, or death, rate of the population as a whole; but when the factor is the selective advantage of one form compared with another, there is no reason why population number should be in any way affected. The treatment of frequency-dependent selection is no clearer; most effects of the relative proportions of model and mimic would conventionally be regarded as density dependent, not frequency dependent. On the other hand, if more than one form of the mimic species were present, frequency-dependent selection would certainly be important; this situation is not discussed.

The shortcomings in the fundamental aspects of selection do less than justice to the author's treatment of evolution at, and above, the species level. Here, one sees, to great advantage, the ad-

mixture of relevant botanical and zoological examples, but it is a surprise to find so little discussion of changes at the chromosomal level; perhaps these are encompassed by neither microevolution nor macroevolution? Recent developments, such as rates of molecular evolution, are also dealt with in a less than satisfactory way, though here the author makes his position clear in one of the many personal statements which add interest to this not uninteresting book.

Are we really to believe (p161) that the decreasing frequency of blood group B, as one passes from central Asia to Western Europe, is a consequence of the decrease in numbers of invading Mongolians as one gets further from Mongolia? And perhaps a little more space might be devoted to the concept (p304) that water-breathing lungs of some fishes were the forerunners of the air-breathing lungs of primitive land vertebrates.

Great care has clearly been taken in the preparation of this volume, but Figs 11.1 and 13.1 would both have been easier to interpret had their respective curves enclosed equal areas. The notation for the haemophilia alleles on p32 seems to have undergone redundant duplication in places. The same could be said of the subject index which, while running to only three pages, has a very large degree of overlap with another index labelled "Key to Technical Terms".

All in all, a very disappointing book which assumes too much for use at an introductory level, but which is inadequate for the more advanced student. □

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Cell population kinetics

An Introduction to Cell Population Kinetics. By W. A. Aherne, R. S. Camplejohn and N. A. Wright. Pp. 88. (Edward Arnold: London, 1977.) £2.95.

This little book provides a very welcome and readable introduction to cell kinetics for the uninitiated. The cell cycle, its constituent phases and the concept of the growth fraction are all

explained, as are the techniques commonly used to measure these parameters. There is a particularly clear exposition of the use of frequency of labelled mitosis (FLM) curves; and various techniques of cell cycle analysis based on continuous labelling and the use of stathmokinetic agents are also explained. There is a chapter on the kinetics of cell populations showing how they can be classified into different

kinetic compartments and how this information is used and there is also a chapter where the ideas introduced in the book are used in worked examples of cytokinetic analysis. Only a knowledge of simple algebra is assumed and so the book should provide an invaluable guide to the analytical methods of cell and population kinetics that are in current usage.

The main fault with this book stems from its main virtue: cell kinetic analysis is not as simple as is made out. An example of this is the interpretation of one of the most commonly used techniques in cell kinetics—the FLM curve. The disparity between the cycle times estimated from the peaks of the FLM curve and those from labelling indices gave rise to the idea that only a fraction of the cells (the growth fraction) was active in proliferation. Perhaps the whole idea of the growth fraction needs re-analysis in view of the observation that continuous labelling with ^3H -thymidine often labels 100% of the cells. Perhaps the growth fraction concept can be dispensed with entirely, if sufficient note is taken of the variation in the cell cycle.

This cell cycle dispersion is often treated as an inconvenient departure

from an ideal. What is only rarely conceded is that this dispersion may be a necessary and unavoidable consequence of the mechanics of the cycle itself. These sort of considerations have edged their way into the subconscious of some cell kineticists who now use computers and sophisticated mathematical models of the cycle in attempts to fit their data. Unfortunately, most of the data is compatible with several kinetic models; the only constant feature being the ability of the theoretical FLM curves to miss most of the experimental points. In spite of this increasing sophistication, as the authors point out, "... to date there is not much evidence that tumour therapy has been made more effective by the use of such data".

Nevertheless, the authors have done a valuable service by explaining in simple terms the rationale behind cell kinetic analysis, although the reader should be aware that, as yet, these concepts are of more heuristic than immediate practical value.

Robert Shields

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Popular survey of visual science

The Miracle of Vision: The Workings and the Wonders of the Human Eye. By A. S. Freese. Pp. 181. (Harper and Row: London, 1977.) £4.50.

THE title and an introduction which includes phrases such as 'the wonders of sight': 'the age of aquarius': and 'the evil eye' identify this text as a short popular survey of visual and medico-visual science. Dr Freese covers in about 200 pages the relevant areas of comparative and neuro-anatomy: perception: visual physiology: the aetiology and correction of refractive errors; and much of historical and modern ophthalmology. This list could be expanded, and following the initial exuberance one could be excused for expecting to find an assortment of inaccuracies such as frequently appear in scientific works designed for the general reader. Surprisingly there are few obvious distortions induced by simplifications, and for this reason it would be unfair to single out every pedantic or minor technical grumble.

I doubt if the lay person is likely to be much interested in so many etymological notes, such as the derivations of chiasm, uveal or circadian. On adaptation it would be proper today to give equal weight to the neuroretinal as well as to the photochemical aspects of visual func-

tion. This book is, however, a series of popular essays, along the lines of the writings of the late A. M. Low, and it is, therefore, a pity that there are so few illustrations and no photographs. Many scientists will find the style subjective and extravagant. Who would risk describing developments in ophthalmological instruments as "... out of the space age's bag of electronic tricks and marvels"?

Dr Freese is subject to no such academic constraints and it is salutary to remember that it is the impersonal, objective presentation of science which is the exception in literature generally.

I have no great argument with the underlying facts, embroidered as they are for lay consumption. But my lasting reservation is whether future reality will match the expectation likely to be aroused by some sections of the work, especially those dealing with the subject of blindness. 'There is even hope for the blind to "see" again', we are told, when indicating recent developments such as electrocortical implants. I accept the 'see' is given in quotes and can include sonar devices, but on this emotive aspect of vision, or lack of it, one should apply the maximum caution when presenting research developments to the public, however exciting, but on which predictions are tenuous at this early stage.

Geoffrey Ball

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Animal learning and human memory

Learning and Memory. By W. A. Wickelgren. Pp. 448. (Prentice-Hall: Englewood Cliffs, New Jersey and Hemel Hempstead, UK, 1977.) \$13.95; £9.75.

THIS clear and lucid text is unusual and unfashionable; the first half presents the field of animal learning and the second that of human memory. This linkage, between areas now commonly kept quite separate, is accompanied by an emphasis on the associative quality of memory in biological systems. Such a point of view makes Pavlov's dogs reasonable neighbours for human beings memorising sentences, and restores the unity of learning lost by most recent texts. The fashionable separation is caused by experimental results suggesting quite different principles in the two cases; for example, hearing the sentence "men eat beef and ducks eat worms" produces no association of the triad "men eat worms" and the links are therefore rather different from those between the stimuli and responses of traditional conditioning. This book deals with such points by 'vertical' association of an item with a more abstract and unobserved entity, rather than with another item. Some such device is reasonable, and indeed inevitable if language and other cognitive functions are to be evolutions of, rather than denials of, simple forms of learning.

Intelligent students will of course notice that tape-recorders or other systems regarded as "non-associative" in this book can similarly be treated as cases of vertical association, and that the emphasis on association seems then to say rather little. They may also comment that facts about animal learning mostly concern reward, punishment, and motivation, while the section on human memory scarcely mentions such points; and this rather weakens the parallelism. Their teachers will notice that, of the topics usually included in human memory, some (surprisingly few) are missing because they fit badly into the general scheme; for instance, many of the factors which alter long-term memory without affecting short-term memory.

The current fashion then has some reason behind it. But it needs challenge, and for those who want to see how far the continuity of animal and human learning can be pushed, this is an effective and engaging survey of the field.

Donald Broadbent

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Environmental dictionary

A Dictionary of the Environment. By Michael Allaby. Pp. 532. (Macmillan: London, 1977.) £12.

THE rapidly evolving vocabulary of any one of 'the life or earth sciences, economics, technology, climatology or meteorology' can be bewildering both to the lay reader and to the specialist trained in another field. Michael Allaby's *A Dictionary of the Environment* attempts to cover them all, and to appeal both to lay reader and scientists alike.

Any good dictionary should be clear, concise, comprehensive, and correct, and Allaby's makes a valiant attempt at being all four. Any failing is due more to the impossible nature of the task than to any want of effort. As he says in his introduction, the "professional ecologist today must be part botanist, zoologist, chemist, physicist, mathematician, geologist, meteorologist, statistician, geographer and, if . . . drawn into the wider environmental movement, economist and philosopher as well".

It is arguably right and proper that in such a work, technical terms should feature at the expense of the wider social vocabulary in which they are used, yet, as a "professional ecologist" himself, Allaby's caps fit best in precisely the order above. Inevitably, the selection of material is arbitrary. "There are omissions, some of them glaring. Economics is covered in a somewhat cursory fashion, some subjects are barely mentioned". And not unexpectedly, herein lies the book's major weakness. Botany, zoology and chemistry are represented well, economics and politics hardly at all. Thus, there are entries for most of the plant families and animal phyla, together with anatomical and physiological terms ("floral formula", "poikilothermy"), whereas neither "fuel cycle" nor "radioactive decay" merit separate entries. The Krebs's cycle is given a full-page diagram, "externalities" a paragraph, but "social cost" is absent, "best practicable means" receives four lines and "blood groups" fourteen.

A few of the entries may be misleading to the uninitiated. The biomass of a lower trophic level is not "always, in a balanced community, higher than that succeeding it", although the annual net production may be. The attentive lay reader might have to think hard about the four-line entry for "uranium" which distinguishes between " $^{238}_{92}\text{U}$ " and " $^{235}_{92}\text{U}$ " without anywhere mentioning the word "isotope"; and to reconcile the statement under "nuclear reactor" that uranium-238 is in its natural form "of no value as nuclear fuel" with that under "magnox" that the "fuel is natural uranium".

The limitations of space to which such over-precise entries may be attributed could perhaps be alleviated by the elimination of the obvious such as "rabbits" ("see Lagomorpha"); and perhaps also the appendix of addresses of UK organisations connected with the environment, which has become almost obligatory in all popular environmental texts. More useful would have been a select bibliography of recent and authoritative introductory texts covering the various disciplines to which difficult entries could refer.

At £12 for just over 500 pages of large print, the price is substantially more than, for example, *The Concise Oxford* with over 1,500 close-typed pages. Perhaps the "students, scientists, conservationists" for whom the *Dictionary* is described on the flyleaf as "an essential tool" might be better off with a collection of the paper-

back specialist science dictionaries plus one of the several (much cheaper, albeit more limited) paperback dictionaries of the environment that have been in print now for some years.

Nevertheless, if you want a comprehensive, hardback dictionary which will take you from "aa" ("Hawaiian term describing basaltic lava with a rough, blocky surface") to "zoochore" ("A plant whose reproductive structures are dispersed by animals") through the "Alkali Inspectorate" and "ZETA" en route, then the *Dictionary of the Environment* will be a useful and worthwhile addition to any library.

Richard Clarke

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Interaction of light with atoms

Atomic and Laser Spectroscopy. By Alan Corney. Pp. 763. (Oxford University Press: London and Oxford, 1977.) £16.

THIS book attempts, fairly successfully, to provide a description of the interaction of light with atoms at a level suitable in the most part for final-year undergraduates, and which incorporates the advances which have so changed the nature of atomic spectroscopy in recent years. The book begins with a review of the basic quantum mechanics and electrodynamics necessary to understand atomic spectra, followed by chapters on spontaneous emission, radiative selection rules and life-times, stimulated emission and absorption, and properties of lasers used in atomic physics. Corney then discusses in some detail resonance fluorescence, double resonance, quantum beats and optical pumping. Each chapter ends with a number of suitable problems and many references for further reading. Certainly the book will be used in advanced undergraduate atomic physics courses. But this is not its only audience: these later chapters are probably the best presentation available of these classic techniques, and will be of great interest to research workers who use or envisage using them.

The preface states that the book is intended for experimentalists. Is such a concept tenable in the late 1970s? Wouldn't theorists gain anything by reading this book, or will they suffer acutely by being forced to recognise real atomic systems rather than models? Equally, are experimentalists so weak-kneed that they need a dilution of theoretical fancies? I

doubt whether an exposure to radiation field quantisation (shunned deliberately in this book) would damage their sensibilities. In many places, I was irritated by the lack of a proper description of the quantum properties of electromagnetic radiation. The author is quite correct to point to the intuitive value of semiclassical theories, but his discussion of the origins of spontaneous emission is particularly weak. The presentation is at too low a level to present some of the successful work using semiclassical theory, and his description of the fully quantised theory is uninformative. Elementary field quantisation is quite within the grasp of final-year undergraduates and certainly within the capabilities of beginning experimental graduate students. Had such a treatment been included, it could only have enhanced the value of this useful book.

In places, more quantitative description would have been useful: for example, the discussion of impact-theory line-broadening falls just short of giving enough information to know how the theory is used in practice. Figure 8.13 clearly exhibits the famous resonance-broadening 'extrapolation anomaly', but this is never explained in the text. It's a pity so much space was taken up on resonant cavity modes, with so little, for example, on multi-photon physics. The book is not too expensive, but is produced by the cheaper typewriter composition method. Despite these faults, the book will be of great value: to undergraduates, to beginning graduate students, even to atomic theorists who aren't afraid of finding out how a laser spectroscopist spends his day.

Peter Knight

Peter Knight is Jubilee Research Fellow in the Department of Physics at Royal Holloway College, University of London, UK.

History and philosophy of psychology

Phenomenology and the Science of Behaviour. By Georges Thines. Pp. 174. (Allen and Unwin: London, 1977.) £8.50.

BECAUSE so many books are simply rehashes of others on the same topic a reviewer must now ask himself: need a particular book have been written? George Thines' book is not a rehash job, for it covers many topics in the history and philosophy of psychology of which most self-respecting Anglo-Saxon psychologists are entirely innocent. This is not to say they should be; after all, educated experimental psychologists ought to be aware of epistemological issues, know what is meant by phenomenology, and understand the strengths and weaknesses of *Gestalt* psychology. All in all, Thines' choice of topics is excellent and their range impressive. It includes, for example, discussions on intentionality, psychoanalysis, dualism and epistemology.

Is there any likelihood, however, that Thines' book will make an inroad into the current robust Anglo-Saxon innocence? His credentials are impressive: he is a Belgian steeped in continental philosophy and experimental psychology and he seems to want to modify the direction of American and British empiricist psychology. But Thines' venture is a difficult one: for a reader asked to change his

intellectual habits has to be persuaded that a different point of view is worthwhile. This was Neisser's brief in *Cognitive Psychology*—a book which made many topics and concepts now current in Anglo-American psychology respectable where before they had been dismissed as too mentalistic. Neisser succeeded brilliantly in persuading psychologists to view their phenomena from an alternative viewpoint because he wrote about their current preoccupations but clarified them from the new vantage point. It is here that Thines is so often weak; he is too abstract and makes little attempt to show that he understands the Anglo-American tradition in psychology and still less does he show that the view from his vantage point would be of help.

Thines' vantage point could illuminate some of the preoccupations this side of the channel but the approach adopted would have to be different. First, there will have to be more of an understanding and respect for our tradition than Thines offers. Second, the treatment of issues will have to be less historical and more concerned with current problems. For example, much of what Thines has to say about intentionality and Brentano could be used to clarify some of the issues which arise in such diverse areas as personality theory, clinical psychology and computer simulation.

William Barnes-Gutteridge

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Machine chess-playing

Chess Skill in Man and Machine. Edited by Peter W. Frey. Pp. 217. (Springer: New York, Heidelberg and Berlin, 1977.) \$16; DM36.20.

"WHY can't a computer be more like a man?"—This technological perversion of Professor Henry Higgins' exasperated outburst must exist in the minds of all those who dabble in machine intelligence. It must also be regarded as the underlying theme of this book, which contains articles concerning the theoretical intentions, and practical outcomes, of those projects which attempt to reproduce human skills in the gladiatorial arena of the chessboard.

Only a few years ago, well informed computer scientists, aware of the nascent explosion in computer hardware capabilities, confidently predicted a master-strength computer chessplayer by 1978. The explosion occurred, but the master program exists only in the minds of the optimistic uninformed. The reality, which comes over forcibly from several different accounts in the book, is that the com-

puters have got bogged down. In spite of their huge calculating power, they cannot produce in five minutes, the quality of moves, or depth of ideas, which the master player produces in five seconds.

Thus, the real problem facing the cybernetic Professor Higgins is revealed, not as the moderate technological problem of computer programming, but as a part of the ancient mystery "What is man?" One can no longer pretend that this problem is straightforward, limited though it is to a small part of man's activities. Indeed, it is just this contact with one of the ultimate questions which makes the problems of machine intelligence so interesting. Can we find out how our own thought processes operate, albeit within closely defined boundaries? Or, in the attempt, can we discover new conceptions in thought, beyond those which the human brain has become accustomed to using?

Well, these problems obviously go beyond the scope of one book. However, for those with a mind to see the current state of affairs in machine chess-playing, and to draw their own conclusions, this book provides a useful starting point.

Nigel Holloway

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Polymer engineering

Treatise on Materials Science and Technology. Vol. 10: Properties of Solid Polymeric Materials. Parts A and B. Edited by J. M. Schultz. (Academic: New York, San Francisco and London, 1977.) A £31.25, \$44; B £24.50, \$34.50.

THE overall aim of this treatise seems to be to provide a review of currently important topics in materials science and engineering for readers who already have a good background knowledge of physics and materials. Volume 10, parts A and B, deals with polymers and includes reviews of microstructures, rubber elasticity, anisotropic elasticity, glassy polymers, fatigue, electronic and electrical properties, and environmental degradation. All but the first are 50–100 pages long, but the chapter on microstructures by J. H. Magill is 350 pages long and could almost stand on its own as a book on polymer crystallisation. Starting from a review of the plastics industry and simple polymerisation and characterisation, Magill discusses crystal structures, morphology and kinetics, matters which are the centre of most polymer crystallisation books. The literature is covered to about the end of 1975.

Two sections which are particularly timely are those on structure-property relationships and on polymer processing. Much of the justification for studying synthetic polymers must be in the use of the results for the improvement of the properties of plastics. It has become accepted in industry that new large scale polymers are unlikely to be produced now that most of the available cheap monomers have been tried, so that the obvious places for improvement are by blending and modifying polymers for better properties or improving processing methods. This places much more emphasis than before on materials science and engineering, a trend which has been supported by the Science Research Council in their establishment of a polymer engineering panel.

Most books on polymer structure make relatively little mention of relationships between physical structure and properties, the whole subject being treated almost as a side issue, whereas processing rarely gets discussed at all. One could argue that in the short run we do not so much need more experiments in polymer engineering as more books which try to bring together polymer science and engineering. Magill has made a worthwhile effort in this direction; it is a pity that the volume is so expensive.

Paul Calvert

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Recent scientific and technical books

Technology

- JAFFEE, Robert I., and WILCOX, Benjamin A. (edited by). *Fundamental Aspects of Structural Alloy Design*. (Battelle Institute Materials Science Colloquia, Seattle, Washington—Harrison Hot Springs, British Columbia, September 15-19, 1975.) Pp.xix + 661. ISBN-0-306-31080-5. (New York and London: Plenum Press, 1977.) \$66.
- KRESSEL, Henry, and BUTLER, J. K. *Semiconductor Lasers and Hetero-Junction LEDs*. (Quantum Electronics: Principles and Applications: a Series of Monographs.) Pp.xiii + 608. ISBN-0-12-426250-3. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$37. £26.25.
- MAGNETIC CIRCUITS AND TRANSFORMERS: a First Course for Power and Communication Engineers. By Members of the Staff of the Department of Electrical Engineering MIT. (Principles of Electrical Engineering Series.) Pp.xvii + 718. (Cambridge, Mass.: The MIT Press, 1977.) \$6.95.
- McMULLAN, J. T., MORGAN, R., and MURRAY, R. B. *Energy Resources*. (Resource and Environment: Sciences Series.) Pp.viii + 177. ISBN-0-7131-5665-5. (London: Edward Arnold (Publishers), Ltd., 1977.) £3.50 paper.
- PALZ, Wolfgang. *Solar Electricity: An Economic Approach to Solar Energy*. Pp.xv + 292. ISBN-0-408-70910-3. (Paris: UNESCO; London: Butterworths, 1978.) £16.
- PAPANTONI-KAZAKOS, P., and KAZAKOS, Dimitri (edited by). *Nonparametric Methods in Communications*. (Electrical Engineering and Electronics: a Series of Reference Books and Textbooks.) Pp.viii + 291. ISBN-0-8247-6660-1. (New York and Basel: Marcel Dekker, Inc., 1977.) \$FR. 80.
- REAY, D. A. *The History of Man-Powered Flight*. Pp.ix + 355. ISBN-0-08-021738-9. (Oxford and New York: Pergamon Press, 1977.) £7.50.
- SEMICONDUCTOR TECHNOLOGY 1977. (Selected papers presented at the 1977 annual SEMINEX Technical Seminar and Exhibition, London, England.) (*Microelectronics and Reliability*, Vol. 16, No. 4, 1977.) Pp.273-526. ISBN-0-08-022148-3. (Oxford and New York: Pergamon Press, 1977.) £9.50.
- SOLAR AGE CATALOG: a Guide to Solar Energy Knowledge and Materials from the Editors of *Solar Age*. Pp.232. ISBN-0-918984-00-9. (Dover, N.J.: Solar Vision, Inc.; Dorchester, Dorset: Prism Press, 1977.) £5.50.
- SPIEGLER, K. S. *Walt-Water Purification*. Second edition. Pp.ix + 189. ISBN-0-306-31030-9. (New York and London: Plenum Press, 1977.) \$23.40.
- SUBRAMANIAN, S. K. *Bio-Gas Systems in Asia*. (Monograph No. 2.) Pp.x + 146. (New Delhi: Management Development Institute, 1977.) Rs. 25; £3; \$5.
- SVAROVSKY, Ladislav (edited by). *Solid-Liquid Separation*. (Chemical Engineering Series.) Pp.xii + 333. (London and Boston, Mass.: Butterworths, 1977.) £21.
- WATSON, J. *Semiconductor Circuit Design for a.f. and d.c. Amplification and Switching*. Third edition. Pp.xi + 536. ISBN-0-85274-324-6. (Bristol: Adam Hilger, Ltd., 1977.) £7.50 net.

Earth sciences

- ANGEL, Martin (edited by). *A Voyage of Discovery*. (George Deacon 70th Anniversary Volume. Supplement to Deep-Sea Research and Oceanographic Abstracts.) Pp.xii + 696. ISBN-0-08-021380-4. (Oxford and New York: Pergamon Press, 1977.) \$45.
- GRIBBIN, John (edited by). *Climatic Change*. Pp.xi + 280. ISBN-0-521-21594-3. (Cambridge, London and New York: Cambridge University Press, 1978.) Hardcover £17.50; Paperback £6.50.
- MENARD, H. W. (with introductions by). *Ocean Science*. (Readings from *Scientific American*.) Pp.307. ISBN-0-7167-0013-1. (San Francisco and Reading, Berks.: W. H. Freeman and Company, 1978.) Hardcover £10.70; Softcover £5.20.
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announcements

Awards

Professor S. W. Hawking of Cambridge University has won the 1978 Albert Einstein Award for outstanding achievement in the study of black holes and superstrong gravitational fields.

The following awards were presented at the New York Academy of Sciences' 160th Annual Meeting: The Presidential Award of The New York Academy of Science to **Professor A. Rich** for his outstanding accomplishments in the field of molecular biology. **H. Shapiro** received The New York Academy of Sciences Award for work in human biology, human evolution and forensic anthropology. To **Professor P. J. E. Peebles**, The Cressy Morrison Award in Natural Sciences for work in astronomy and cosmology. **D. B. Brown** received the Boris Pregel Award for Research in Biology. **Professor S. Coleman** won the Boris Pregel Award for Research in Nuclear Physics and Nuclear Engineering. **Professor J. D. Cole** received the I. B. Laskowitz Award for Research in Aerospace Engineering Sciences, Support Systems, and Components. **Professor F. S. Rowland** was presented with the Gordon Y. Billard Award for Research in Environmental Sciences. **Professor W. Gilbert** received the Louis and Bert Freedman Foundation Award for Research in Biochemistry. **Professor I. Tamm** received the Sarah L. Pooley Memorial Award for his work on understanding of the mechanisms of RNA virus replication, and also on controlling influenza virus infection. **Professor R. K. Crane** won the Dr Harold Lampert Award for excellence in research and teaching in physiology. **Professor N. Turro** received the Freda and Gregory Halpern Award in Photochemistry.

The Zoological Society of London has made the following awards for contributions to zoology in 1977; The Scientific Medal—to **Dr D. W. T. Crompton** (Molteno Institute of Biology and Parasitology, University of Cambridge); **Dr R. L. Gardner** (Department of Zoology, University of Oxford); and **Dr P. A. Lawrence** (MRC Laboratory of Molecular Biology, Cambridge). The Stamford Raffles Award to **S. Cramp**. The Thomas Henry Huxley Award to **Dr A. Knox** (University of Aberdeen). The Zoological Society of London Frink Medal for British Zoo-

logists to **Dr S. M. Manton, FRS** (Honorary Associate, British Museum (Natural History)). The Prince Philip Prize to **P. J. Azzopardi** (Stonyhurst College).

The ARC have reviewed their policy on the Wain Trust Fund which provides grants for facilitating the interchange of scientists between universities in the UK and universities overseas, and can now offer up to six Wain Fellowships annually to enable younger research scientists to work or study abroad at an academic, industrial or agricultural institution of their choice for a period of up to three months. The closing dates for applications are 1 April and 1 October annually. Further particulars from Secretary, ARC, 160 Portland Street, London W1.

A new professional qualification, the Mastership in Food Control, was launched on 1 January. It will be awarded on the results of comprehensive written and oral examination. The first examinations will take place in London in September 1978. Contact the Institute of Food Science and Technology, MFC Section, 105-110 Euston Street, London NW1.

Appointments

Dr W. J. Peacock succeeds Dr L. T. Evans as Chief of CSIRO's Division of Plant Industry in Canberra.

Professor Charlotte Friend has become the first woman president of the New York Academy of Sciences.

Professor Kurt G. M. M. Alberti to the Chair and Headship of the Department of Clinical Biochemistry at the University of Newcastle upon Tyne, from October 1978.

Professor B. K. Follett to the Chair and Headship of the Department of Zoology at the University of Bristol.

Person to person

W. Lister would like to hear from anyone with historical information preferably pre-1910 on the Muirhead instrument firm. Contact him at 1 Engel Park, Mill Hill, London NW7.

Chemist seeks 4/5 bedroom house in York July 1978 to July 1979, rent or exchange. Contact Prof. M. Laing, Department of Chemistry, University of Natal, Durban 4000, South Africa.

On the move

Dr A. G. Kitchell from Chief Scientist's Group, Ministry of Agriculture, Fisheries and Food to Head of Scientific Services and Development Division at the Food Research Institute, Colney Lane, Norwich.

P. H. Frampton from U.C.L.A. to Department of Physics, Ohio State University, Columbus, Ohio 43210.

Meetings

5-6 April, **Acceptable Risk**, London (Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).

5-6 April, **Workshop on the Role of Planning in the Control of Environmental Pollution**, Bristol (Jane Dumore, NSCA, 136 North Street, Brighton, Sussex, UK).

7-8 April, **10th Stadler Genetics Symposium**, Missouri (Stadler Genetics Symposia, 117 Curtis Hall, University of Missouri, Columbia, Missouri 65201).

10-12 April, **Crystallography in Materials Science**, Bath (Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1, UK).

10-14 April, **Medicinal Chemistry**, Bradford (Sara Urwin, The Chemical Society, Burlington House, London W1, UK).

10-14 April, **IXth International Symposium on Carbohydrate Chemistry**, London (Dr John Gibson, The Chemical Society, Burlington House, London W1, UK).

13 April, **Diffusion and Transport Processes related to Biological and Environmental Processes**, London (Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).

16-20 April, **7th International Geochemical Exploration Symposium**, Colorado (M. A. Chaffee, 7th IGES, US Geological Survey, 5946 McIntyre Street, Golden, Colorado 80401).

13-14 April, **Enzymatic and Non-Enzymatic Catalysts**, London (Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).

8-11 May, **49th Annual Scientific Meeting of the Aerospace Medical Association**, New Orleans (Aerospace Medical Association, Washington National Airport, Washington, DC 20001).

15-17 May, **9th International Symposium on Chromatography and Electrophoresis**, Riva del Garda (Dr Alberto Frigerio, Istituto di Recerche Farmacologiche "Mario Negri" Vie Eritrea, 62-20157 Milano, Italy).

21–24 May, **1st International Symposium on Radiopharmacology**, Innsbruck (Dr Lelio G. Colombetti, Pharmacology Department, Loyola University Stritch School of Medicine, Maywood, Ill. 60153).

22–26 May, **8th International Meeting of the International Association of Forensic Sciences**, Kansas (Secretariat, I.A.F.S., PO Box 8282, Wichita, Kansas 67214).

22–23 May, **International Symposium on Corrosion and Degradation of Implant Materials**, Kansas (Peter Brown, Standards Development Division of ASTM, 1916 Race St, Philadelphia, Pasadena 19103).

21–24 May, **13th Congress of European Society for Surgical Research**, Helsinki (ESSR 1978, Travel Experts Ltd, PO Box 722, SF-00101 Helsinki 10, Finland).

23–26 May, **7th European Conference on Macromolecular Physics**, Strasbourg (Henri Benoit, 7th EPS Conference, Centre de Recherches sur les Macromolécules, 6 rue Boussingault, 67083 Strasbourg, Cedex, France).

3–5 July, **Annual Meeting of the European Tissue Culture Society**, Glasgow (as soon as possible, Dr Ian Freshney, Beatson Institute for Cancer Research, Bearsden Road, Glasgow, UK).

Reports & Publications

UK & Ireland—December

Safety in Laboratories. Pp. 42. (Simonsway, Manchester M22: Group Information Department, CIBA-GEIGY (UK), Ltd., 1977.) 25p. [112]

Ant Research 1954–76. By Michael Brian, Andrew Abbott, Barrie Pearson and Judith Wardlaw. (Natural Environment Research Council.) Pp. 27. (Cambridge: Institute of Terrestrial Ecology, 68 Hills Road, 1977.) £1.20 net. [121]

Third Report of the DHSS/SSRC Joint Working Party on Transmitted Deprivation. Pp. 12 + vii. (London: Social Science Research Council, 1 Temple Avenue, EC4, 1977.) [121]

Amnesty International Report for 1977. Pp. 352. (London: Amnesty International Publications, 10 Southampton Street, WC2, 1977.) £2. [151]

International Planned Parenthood Federation. Occasional Essays. No. 2: Population and National Development—the Dilemma of Developing Countries. By Fred T. Sai, with editorial assistance from Penny Kane. Pp. 31. No. 3: Food, Pollution and Politics. By Fred T. Sai. Pp. 36. 85p. No. 4: Defining Family Health Needs, Standards of Care and Priorities, with particular reference to Family Planning. By Fred T. Sai. Pp. 32. 85p; \$1.45. No. 5: Health, Nutrition and Population in Human Settlements. By Fred T. Sai. Pp. 32. 85p; \$1.45. (London: International Planned Parenthood Federation, 18–20 Lower Regent Street, SW1, 1977.) [612]

International Union of Pure and Applied Chemistry: Physical Chemistry Division. Commission on Physicochemical Measurement and Standards: Subcommittee on Calibration and Test Materials. Recommended Reference Materials for Realization of Physicochemical Properties. Section: Pressure-Volume-Temperature Relationships. Collator: D. Ambrose. Pp. 1437–1464. (Oxford and New York: Pergamon Press, 1977.) £3.30. [712]

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Department of Industry. Life-cycle Costing in the Management of Assets: a Practical Guide. Pp. viii + 57. (London: HMSO, 1977.) £1.75 net. [812]

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Nature Conservancy Council. Third Report covering the Period 1 April, 1976–31 March, 1977. Pp. viii + 117 + 10 plates. (London: HMSO, 1977.) £3.10 net. [1212]

The University of Hull. Annual Report, 1976/1977. Pp. xii + 116. (Hull: The University, 1977.) [1412]

Proceedings of the Royal Irish Academy. Vol. 77, A, No. 5: Finite Groups Generated by a Pair of Unitary Matrices with Normal Sum. By T. J. Lalley. Pp. 61–74. (Dublin: Royal Irish Academy, 1977.) 98p. [1412]

British Overseas Trade Board. Report for 1977. Pp. 48. (London: British Overseas Trade Board, 1 Victoria Street, SW1, 1977.) [1412]

University of Oxford. Annual Report, 1976–77. Pp. 22. (Supplement No. 1 to the *University Gazette*, Vol. CVIII, Dec. 1977.) (Oxford: The University, 1977.) £1.25. [1512]

Department of Health and Social Security. Department of Education and Science. Scottish Office. Welsh Office. Prevention and Health. Pp. vi + 85. (London: HMSO, 1977.) £1.60 net. [1612]

Potato Marketing Board. Commercial Assessment of Recently Introduced Potato Varieties—Report on Trials and Surveys 1977. Collated by R. M. Meredith. Pp. 28. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1977.) 25p. [1912]

Cabinet Office. Government Research and Development: a Guide to Sources of Information. Pp. iv + 39. (London: HMSO, 1977.) 85p. [1912]

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Proceedings of the Royal Irish Academy. Section B: Biological, Geological and Chemical Science. Vol. 77, B, No. 9: Large *Eunice harassii* Audouin and Milne-Edwards from a Sludge Disposal Area. By A. J. M. Walker. Pp. 175–179. (Dublin: Royal Irish Academy, 1977.) 60p. [2112]

Ministry of Agriculture, Fisheries and Food: Directorate of Fisheries Research. Library Information Leaflet No. 6: The Effects of Photoperiod on Reproduction in Fishes—An Annotated Bibliography. By M. Hün-Han. Pp. 30. (Lowestoft: Ministry of Agriculture, Fisheries and Food, Fisheries Laboratory, 1977.) [2112]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 287, No. 1347: Tidal Dissipation on the Oceans—Astronomical, Geophysical and Oceanographic Consequences. By K. Lambeck. Pp. 545–594. UK £3; Overseas £3.10. Vol. 287, No. 1348: The Sabaloka Igneous Complex, Sudan. By D. C. Almond. Pp. 595 + 633 + pull out map. UK £2.65; Overseas £2.75. (London: The Royal Society, 1977.) [2212]

Natural Environment Research Council. Annual Report of the Institute of Terrestrial Ecology for 1976. Pp. vii + 99. (London: HMSO, 1977.) £3.50 net. [2212]

Ministry of Overseas Development. Report on Research and Development 1977. Pp. 76. (London: HMSO, 1977.) £1.60 net. [2212]

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Department of Health and Social Security. Health and Personal Social Services Statistics for England (with summary tables for Great Britain), 1977. (A publication of the Government Statistical Service.) Pp. 206. London: HMSO, 1977.) £6 net. [3012]

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Bibliography of Society, Ethics and the Life Sciences: Supplement for 1977–78. Full Bibliography compiled by Sharon Solitto and Robert M. Veatch. Supplement compiled by Nancy K. Taylor. Pp. 36. (Hastings-on-Hudson: Institute of Society, Ethics and the Life Sciences, The Hastings Center, 1977.) [1112]

National Institutes of Health. Environmental Impact Statement on NIH Guidelines for Research Involving Recombinant DNA Molecules, Parts 1 and 2. (Bethesda, Md.: US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, 1977.) [2112]

United States Department of the Interior: Geological Survey. Professional Paper 1015: Proceedings of the First Annual William T. Pecora Memorial Symposium, October 1975, Sioux Falls, South Dakota. Edited by P. W. Woll and W. A. Fischer. Pp. xxv + 370. (Washington, DC: US Government Printing Office, 1977.) [2112]

World Health Organization. Public Health Papers, No. 67: Health Services—Concepts and Information for National Planning and Management. (Experiences Based on the WHO/International Collaborative Study of Medical Care Utilization.) By Kerr L. S. White *et al.* Pp. 117. (Geneva: WHO; London: HMSO, 1977.) Sw. fr. 11. [512]

Animal Regulation Studies, Vol. 1, No. 1, August 1977. Editor-in-Chief: Dr W. Ross Cockrill. Published quarterly. Pp. 1–102. Subscription price for volume 1 is Dfl. 117.00; £47.75. (Amsterdam: Elsevier Scientific Publishing Company, 1977.) [612]

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Pakistan Association for the Advancement of Science. Its History and Achievements, 1947–1975. By M. I. D. Chughtai. Pp. viii + 95. (Lahore: Pakistan Association for the Advancement of Science, 14, Shah Jamal Colony, 1977.) \$5. [612]

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